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Effect of adding fermented shrimp waste extract in ration on metabolizable energy and nitrogen retention in laying hens

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Abstract

The study aims to determine and obtain the level of administration of fermented shrimp waste extract on the metabolizable energy value and nitrogen retention in laying hens of the production phase. The method used was experimental with complete randomized design and the effect of treatment using fingerprints with the Dunnet test. The treatment consists of five kinds with four repetitions, namely R0, the ration does not contain fermented shrimp waste extract; R1, ration contains 0.5% fermented shrimp waste extract; R2, ration contains 1% fermented shrimp waste extract; R3, the ration contains 1.5% fermented shrimp waste extract; R4, ration contains 2% fermented shrimp waste extract. The results showed that the addition of fermented shrimp waste extract as much as 1.5% resulted in the highest metabolizable energy value, and the addition of 1% resulted in the highest nitrogen retention value.

Keywords: Fermented shrimp waste extract; Laying hens; Metabolizable energy; Nitrogen retention

1. Introduction

A ration is a mixture of several feed ingredients that are mixed to meet the various nutrients needed by laying hens. Ration is one of the important components in laying hens, one of which is for egg production. Egg production can be optimal if the ration is in accordance with the nutritional needs of laying hens. The quality and quantity of feed given greatly affects the productivity and quality of eggs. Productivity will be achieved efficiently if the feed provided is sufficient for the needs of chickens in accordance with age and maintenance management [1].

The productivity of laying hens is inseparable from the quality of the feed given. Laying hens consume feed to meet their protein and energy needs. Feeding rations with good protein quality will certainly affect the growth rate and development of chickens. Protein quality is determined by the feed ingredients that make up the ration, especially in protein source feed ingredients that have better nutrient content.

Feeding additives needs to be done with the aim of achieving efforts to efficiently use rations. Affixes are intended to spur growth or improve livestock productivity and health and increase production efficiency. Feed additives are very commonly used in the modern livestock industry that are mixed into rations that can affect the efficiency of nutrient use [2].

Shrimp waste fermentation products are made from shrimp waste left over from shrimp processing after taking the meat part, so that what is left is the head, shell, tail, and small shrimp intact in small quantities. The quality and nutrient

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content of shrimp waste is highly dependent on the proportions of the shrimp head and shell [3]. Fermented shrimp waste products have a crude protein content of 39.29%, crude fat 7.03% and crude fiber 6.81% [4].

One of the ingredients that can be used as feed supplements in poultry rations is fermented shrimp waste extract. Shrimp waste contains protein and minerals as well as astaxanthin which illustrates the potential to be used as a feed affix in poultry rations. The content of astaxanthin in fermented shrimp waste extract is 436 ppm [5].

Astaxanthin is a carotenoid compound with such a structure that makes it active as an antioxidant [6]. Astaxanthin in shrimp waste shows strong antioxidant activity [7]. Shrimp waste has weaknesses as feed because it contains chitin which causes protein and minerals (in the form of calcium carbonate) to be bound very strongly and are difficult to digest. Chitin is a biopolymer of N-acetyl-D-glucosamine units of white, tasteless, odorless, and water-insoluble, generally organic solvent, inorganic acid, and dilute base [8]. Chitin binds to the N of the amino acids that make up protein so that protein becomes difficult to digest. One way to decompose chitin is through biological processes or with chitinase enzymes that can hydrolyze chitin polymer compounds into chitin oligosaccharides (N-acetyl glucosamine monomers) [9]. Chitinase enzyme is an enzyme responsible for degrading chitin, capable of producing cellulase into glucose [10]. Poultry is a type of livestock that cannot produce chitinase enzyme in the digestive tract. Therefore, it is necessary to try to improve its quality so that it can be used as feed ingredients in the preparation of poultry rations, one of which is by fermentation, because fermentation treatment can help poultry digest feed ingredients containing chitin [11].

The process of chitin fermentation in shrimp waste as a limiting factor is carried out through two stages, namely deproteination using *Bacillus licheniformis,* demineralization with *lactobacillus* sp*.* and bioprocessing with *Saccharomyces cerevisiae.* These three microorganisms are bacteria capable of producing proteases and chitinase in relatively high quantities [12]. *Lactobacillus* sp. is a microbe that breaks down glucose, sucrose, maltose, and lactose into lactic acid resulting in mineral deposits [13]. Shrimp waste fermented using *B. licheniformis bacteria, Lactobacillus* sp*.* and *S. cerevisiae* extracted to be mixed in rations.

Nitrogen retention and metabolizable energy is one method to assess protein quality and energy content of rations. Metabolizable energy is the difference in the gross energy content of rations released through excreta. Metabolizable ally corrected nitrogen energy (MEn) is a measure of the energy used by livestock for body metabolism and growth, which has been corrected for nitrogen produced by livestock in urine and feces. In the context of livestock nutrition, MEn are used to calculate the energy value of food consumed by animals, and can be used to make more precise and efficient ration formulations for farm animals.

MEn measurements are more accurate than gross metabolizable energy (ME) measurements because ME does not consider the energy used to remove nitrogen in urine and feces. Therefore, MEn measurement is important in determining the energy needs of farm animals and formulating rations that suit their nutritional needs.

The less energy expended, the higher the ration energy absorbed or digested by the body, so the efficiency of ration energy use is high [14]. Nitrogen retention is the amount of nitrogen in feed protein that can be retained and used by the livestock body [14]. The calculation of the nitrogen retention value is taken from the amount of nitrogen minus the amount of nitrogen in excreta and urine. The more nitrogen retained in the poultry's body, the smaller the amount of nitrogen in excreta and urine [15]. The nutrient content in feed ingredients that are important to note is protein. Protein is the main element of the body and soft tissues of various poultry. They are necessary for growth, management and egg production and are part of all enzymes in the body [16]. The efficiency of protein use is shown by nitrogen retention, this is because the more nitrogen retention is retained, the more protein is absorbed, because nitrogen retention and metabolizable energy are one of the decisive factors in livestock productivity [17].

Astaxanthin concentrations in the range of 10-20 μg/ml indicate a level of antioxidant activity [18]. Addition of probiotics (*Lactobacillus sp.*) in feed at the level of 0.6% gives results on metabolizable energy and N collated metabolizable energy in quail [19]. The results of another study showed that the addition of 1.17% dahlia tuber extract in the ration had a significant effect on metabolizable energy value and nitrogen retention value in local chickens [20]. Additional feeding in rations ranging from 0.6% to 1.17% affects metabolizable energy value and nitrogen retention.

2. Material and methods

2.1. Experimental Livestock

The study used 20 laying hens of the Lohmann phase layer phase as many as 20 heads. Chickens are divided into 5 types of treatment and repeated 4 times.

2.2. Trial Cage

The cage used is an individual-shaped cage of 20 units with a length of 40 cm, a width of 30 cm, and a height of 35 cm made of bamboo. Each cage unit is filled with 1 chicken.

2.3. Feed Ingredients Constituent of Rations

The feed ingredients that make up the ration consist of yellow corn, soybean meal, meat bone meal, coconut oil, bone meal, stone meal, grit, lysine, and methionine. Feeding is carried out 2 times a day in the morning and evening with the amount of each feeding 50 grams / head. The nutrient content of ration constituent materials can be seen in Table 1.

Table 1 Metabolizable Energy Content and Nutrients of Experimental Feed Ingredients

Feed Ingredients	MEn	CP	EE	CF	Ca	P	Lys.	Meth.
	(Kcal/kg)	..... (%).....						
Yellow corn	3350	8.60	3.80	2.20	0.02	0.08	0.26	0.18
Soybean meal	2230	44.00	0.80	7.00	0.29	0.27	2.69	0.62
Fermented shrimp waste extract	3033	25.15	0.96	-	6.81	2.83	0.85	0.28
Meat bone meal	2375	38.84	10.93	2.46	9.80	4.50	2.08	0.54
Coconut oil	8600	-	-	-	-	-	-	-
Bone meal	-	-	-	-	24.00	12-00	-	-
Stone flour	-	-	-	-	40.00	-	-	-
Grit	-	-	-	-	30.87	1.11	-	-

Source: Analysis results of the Ruminant Animal Nutrition and Fodder Chemistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University (2022)

2.4. Research Ration Arrangement

Table 2 Trial Ration Formulation

No	Feed Ingredients	R0	R1	R2	R3	R4
		..... (%).....				.....
1	Yellow corn	58.10	57.70	57.50	57.30	57.10
2	Soybean meal	21.00	20.90	20.60	20.30	20.00
3	Fermented shrimp waste extract	0.00	0.50	1.00	1.50	2.00
4	Meat bone meal	8.50	8.50	8.50	8.50	8.50
5	Coconut oil	1,00	1.00	1.00	1.00	1.00
6	Bone meal	3.15	3.15	3.15	3.15	3.15
7	Stone flour	4.50	4.50	4.50	4.50	4.50
8	Grit	3.75	3.75	3.75	3.75	3.75

Remarks: R0, ration does not contain fermented shrimp waste extract; R1, ration contains 0.5% fermented shrimp waste extract; R2, ration contains 1% fermented shrimp waste extract; R3, ration contains 1.5% fermented shrimp waste extract; R4, rations contain 2% fermented shrimp waste extract

The ration is prepared based on the standard requirements of protein content and metabolizable energy according to *Hy-line* international (2016). The arrangement of experimental rations is presented in Table 2.

Table 3 Metabolizable Energy Content and Nutrients of Experimental Rations

Nutrient Content	R0	R1	R2	R3	R4	Necessity*
ME (kcal/kg)	2.703	2.702	2.704	2.706	2.707	2.700-2.750
Crude protein (%)	17.54	17.59	17.56	17.54	17.52	17.50
Crude fat (%)	3.93	3.92	3.91	3.89	3.88	3-4
Crude fibre (%)	2.96	2.94	2.92	2.89	2.87	≥2.60
As (%)	4.62	4.65	4.69	4.72	4.75	≥4.30
P (%)	0.91	0.92	0.93	0.95	0.96	≥0.80
Lysine (%)	0.89	0.89	0.89	0.88	0.88	≥ 0.88
Methionine (%)	0.52	0.52	0.52	0.52	0.51	≥ 0.48

Remarks: *Needs based on Hyaline International (2016)

2.5. Research Procedure

The chicken is weighed first to find out its initial weight. Chickens were randomly coded at each treatment and repeated experiments to avoid errors during the study, each cage was coded on the front of the cage by forging a coded label.

The implementation of the study was carried out for 10 days. Habituation of feed is carried out in the first 7 days. Day 8 to day 10 observations were made. The amount of ration given is 100 grams / head / day and the provision of drinking water is carried out ad libitum.

Feed consumption is considered by recording the difference between the amount of feed given and the amount of feed left. Excreta storage is carried out for 24 hours accommodated using plastic trays, taken every morning and then weighed and recorded as wet weight. Excreta that have been weighed and recorded as wet weight and then dried by drying and weighing as dry weight.

2.6. Observed Modifiers

2.6.1. Metabolizable energy (AMEn)

Metabolizable energy is calculated by method [22] with the following formula:

$$\text{MEn} = \frac{(\text{Ebr x K}) - (\text{Je x Ebe}) - \left\{\frac{\text{K X Nr}}{100}\right\} - \left\{\frac{\text{Je x Ne}}{100}\right\} \text{x } 8{,}22}{\text{K}}$$

Information:
MEn : Metabolizable energy corrected by nitrogen retention (kcal/kg)
Ebr : Gross Energy Ration (kcal/kg)
K : Number of Rations Consumed (kg)
Je : Amount of Excreta (kg)
Ebe : Gross Energy of Excreta (kcal/kg)
Nr : Nitrogen Ration (%)
Ne : Excreta Nitrogen(%)
8.22: constant value of metabolizable energy retained

2.6.2. Nitrogen Retention

The determination of nitrogen retention value is calculated by method [21] with the following formula:

$$\text{RN (\%)} = \frac{\text{NI} - \text{NF}}{\text{NI}} \; X \; 100\%$$

Information:
NI : Nitrogen Consumption (g)
NF : Excreta Nitrogen (g)

2.7. Statistical Analysis

Statistical analysis to determine the effect of treatment on observed variables was carried out using the fingerprint method [23]. The mathematical model of the experimental design is:

$$Yij = \mu + \alpha i + eij$$

Information:

Information:
Yij = response to the i-treatment of the j-th repetition
μ = Middle value of population
αi = Effect of i-th treatment
eij = Effect of error or random trial (error attempt)

In the I-th treatment, the J-th test.

If the results of the analysis obtained are significantly different, then further tests are carried out using the Dunnet test, with the following mathematical formula:

$$d = t(\text{Dunnet}) \left(2\frac{KTG}{r}\right)^{1/2}$$

Decision Rules:
$|X_i - X_0| \leq d$, The treatment is not significantly different
$|X_i - X_0| \geq d$, Real different treatment

3. Results and discussion

3.1. Effects of Treatment on Metabolizable Energy in Laying Hens

Metabolizable energy is the difference in the gross energy content of the ration with the gross energy expended through excreta. The results of variance analysis from a complete randomized design showed that the use of fermented shrimp waste extract in the ration had a significant effect on metabolizable energy. This can illustrate that the administration of fermented shrimp waste extract gives a positive response to laying hens to the amount of energy metabolized by the chicken's body. Dunnet's test results can be seen in Table 4.

Table 4 Dunnet Test Effects of Treatment on Metabolizable Energy

Execution		Track	Value d	Different Absolute
R1	R0	2.400	185.56	50.09b
R2	R0	2.992	185.56	641.39a
R3	R0	3.400	185.56	1.049.95a
R4	R0	2.843	185.56	492.37a

Description: a, different (P<0.05) b, not different

Based on the results of the Dunnet test in Table 4, the metabolizable energy value (ME) of laying hen rations in R2, R3, and R4 treatment showed significant results with R0 control treatment. The R1 treatment did not show a significant difference with the R0 control treatment. Basal rations added 0.5-2% fermented shrimp waste extract produced higher metabolizable energy than control rations, but based on Dunnet's test, only 1-2% fermented shrimp waste extract was added which provided significant treatment, namely R2, R3 and R4 treatment while R1 did not provide significant

treatment to R0 control rations. This showed that the addition of shrimp waste extract by 1-2% gave better results than the control ration and the addition rate of 0.5% fermented shrimp waste extract gave the same good results as the control ration.

The increase in metabolizable energy value in each treatment is due to the addition of feed supplements in the ration formulation of laying hens. This means that the addition of fermented shrimp waste extract in the ration can help streamline the energy metabolism of laying hens to increase the metabolizable energy value in laying hens. This shows that there is an improvement in the nutritional quality of laying hen rations due to the use of feed supplements from fermented products. Opinion [5] states that fermentation products from deproteination by *B. licheniformis* followed by mineralization by *Lactobacillus* sp. and S. *cerevisiae* has better metabolizable energy value and protein digestibility. This is because *B. licheniformis* is a bacterium that can produce protease enzymes and chitinase in relatively high quantities and an acidic atmosphere formed by *Lactobacillus* sp. allowing minerals bound to proteins to be released, the fermentation process with *S. cerevisiae* can improve digestibility through the process of carbohydrate enzymes and proteases. According to [24] the fermentation process causes changes in the properties of feed ingredients as a result of the breakdown of food content by enzymes produced by microbes, feed ingredients resulting from the fermentation process have better nutritional value than the original ingredients, this is evident from the difference in gross energy and protein content in basal rations or control rations with rations that have been given fermented shrimp waste extract.

Shrimp waste contains high astaxanthin, the astaxanthin content contained in fermented shrimp waste extract is 436 ppm [5]. Astaxanthin is a carotenoid compound that is active as an antioxidant. Opinion [7] states that astaxanthin in shrimp waste shows strong antioxidant activity, so that it can suppress the growth of pathogenic bacteria contained in the intestine and improve intestinal villi to maximize the digestibility of food substances from the feed consumed. This is reinforced by the opinion [25] that the decrease in the population of pathogenic bacteria has a positive effect on increasing the metabolizable energy of chickens because it reduces the competition between pathogenic bacteria and their hosts in utilizing energy from feed that enters the digestive tract. The value of metabolizable energy is directly proportional to digestibility, the increase in digestibility value in chicken then the metabolizable energy value will increase as well. Opinion [26] that the high digestibility value is influenced by the improvement of feed quality, which can also affect the increase in metabolizable energy. In addition, [27] states that metabolizable energy is influenced by the gross energy content of the feed and the amount of energy used by the livestock itself.

The highest metabolizable energy value in this study was obtained by R3 treatment with the addition of 1.5% fermented shrimp waste extract to the ration of laying hens, which was 3400 kcal / kg. This result is different from research 26) which states that the use of feed supplements based on fermented shrimp waste extract by 5% provides the highest metabolizable energy value in broiler chickens. The difference in metabolizable energy value is caused by the difference in the level of use of feed supplements in laying hens.

3.2. Effects of Treatment on Nitrogen-Corrected Metabolizable Energy in Laying Hens

Nitrogen-corrected metabolizable energy is the nitrogen-corrected metabolizable energy value, which is the result of reducing the calorific value of one gram of nitrogen (8.22) multiplied by nitrogen retention. The results of the fingerprints showed that the provision of shrimp waste in the ration had a real effect ($P<0.05$) on the nitrogen-corrected metabolizable energy value. To see the difference in the effect between treatment and control, the Dunnet test was carried out. The test results can be seen in Table 5.

Table 5 Dunnet Test Effect of Treatment on Corrected Metabolizable Energy Nitrogen

Execution		Track	Value d	Absolute Difference
R1	R0	2.383	184.43	47.39[b]
R2	R0	2.973	184.43	636.94[a]
R3	R0	3.382	184.43	1,046[a]
R4	R0	2.824	184.43	488.21[a]

Description: a, different ($P<0.05$) b, not different

Based on the results of the Dunnet test in Table 5, the nitrogen-corrected metabolizable energy value of laying hen rations in R2, R3, and R4 treatments showed significant results with R0 control treatment. The R1 treatment did not show a significant difference with the R0 control treatment. Basal rations added 0.5-2% fermented shrimp waste extract

produced higher nitrogen-corrected metabolizable energy than control rations, but based on Dunnet's test, only 1-2% addition of fermented shrimp waste extract provided significant treatment, namely R2, R3 and R4 treatment while R1 did not provide significant treatment of R0 control rations. This showed that the addition of shrimp waste extract by 1-2% gave better results than the control ration and the addition rate of 0.5% fermented shrimp waste extract gave the same good results as the control ration.

The nitrogen-corrected metabolizable energy value is influenced by gross energy consumption and crude protein from feed, protein quality, nitrogen consumption, and food balance in feed. The MEn value obtained shows the metabolizable energy value which is further corrected by the nitrogen retention value, namely by subtracting the calorific value of 1 gram of nitrogen (8.22) then multiplied by nitrogen retention so that the value is always lower than the metabolizable energy. Nitrogen retention indicates the amount of protein left behind in the body. According to [28] the quality of protein is low or one of the amino acids in a feed ingredient is less then nitrogen retention will be low. Opinion [29] that the results of calculating the metabolizable energy of feed without nitrogen correction are considered to underestimate the energy value of a feed because nitrogen stored in body tissues retained nitrogen, when catabolized the result will be expressed as energy lost as urine. With the calculation of corrected metabolizable energy, nitrogen is expected to be unaffected by nitrogen.

The comparative value of metabolizable energy and nitrogen-corrected metabolizable energy is as follows: in R1 the value of metabolizable energy is 2400 kcal / kg after correction with the value of nitrogen retention reduced to 2383 kcal / kg of energy lost in R1 which is 16.92 kcal / kg, then in R2 the ME value of 2992 kcal / kg after correction with the value of nitrogen retention to 2973 kcal / kg of energy lost is 18.67 kcal / kg, then in R3 the ME value was 3400 kcal / kg after correction with a nitrogen retention value to 3382 kcal / kg of lost energy of 17.87 kcal / kg, then in R4 the ME value of 2843 kcal / kg after correction with a nitrogen retention value to 2824 kcal / kg of lost energy of 18.38 kcal / kg. Based on the comparative value of ME and MEn the order of energy loss is largest to smallest in R2 treatment: 18.67 kcal / kg; R4: 18.38 kcal/kg; R3: 17.87 kcal/kg; and R1: 16.92 kcal/kg.

3.3. Effects of Treatment on Nitrogen Retention in Laying Hens

Nitrogen retention is the amount of nitrogen in the protein ration that is absorbed and used by the body of livestock. The results of variance analysis from the complete randomized design showed that the use of fermented shrimp waste extract in the ration had a significantly different effect ($P<0.05$) on nitrogen retention. Dunnet's test results can be seen in Table 6.

Table 6 Dunnet Test Effects of Treatment on Nitrogen Retention

Execution		Track	Value d	Absolute Difference
R1	R0	65.75	6.84	6.10[b]
R2	R0	71.85	6.84	12.20[a]
R3	R0	68.95	6.84	8.94[a]
R4	R0	66.74	6.84	7.09[a]

Description: a, different ($P<0.05$) b, not different

Based on the results of the Dunnet test in Table 6, the nitrogen retention values of laying hen rations in R2, R3, and R4 treatments showed significant results with R0 control treatment. The R1 treatment did not show a significant difference with the R0 control treatment. Basal rations added 0.5-2% fermented shrimp waste extract resulted in higher nitrogen retention values than control rations, but based on Dunnet's test only the addition of 1-2% fermented shrimp waste extract provided significant treatment, namely R2, R3 and R4 treatments) while R1 did not provide significant treatment to the R0 control ration. This showed that the addition of shrimp waste extract by 1-2% gave better results than the control ration and the addition rate of 0.5% fermented shrimp waste extract gave the same good results as the control ration.

A nitrogen consumption value greater than the excreted nitrogen indicates that the nitrogen retention value is positive, while nitrogen retention is negative if the nitrogen consumed is smaller than the excreted nitrogen. The value of nitrogen retention has a real effect on this research because rations given fermented shrimp waste extract have improved nutritional quality. The fermentation process affects the protein digestibility rate of a feed, this is in accordance with opinion [5] that fermentation products from deproteination by *B. licheniformis* followed by mineralization by *Lactobacillus* sp. and *S. cerevisiae* have better metabolizable energy value and protein digestibility.

This is because the fermentation process by *S. cerevisiae* can improve digestibility through the production of enzymes and proteases. Factors that affect nitrogen retention are protein consumption, protein quality, protein digestibility and nutrients in the ration. In addition, factors that affect the size of the nitrogen retention value are the energy level in the ration and the condition of the livestock itself.

The nitrogen retention value in this study was 66.42% higher than the study conducted by [30] with the average nitrogen retention value of Sentul chickens aged 12 weeks reaching 63.25%. According to [31], the large amount of protein that can be absorbed by the body results in the chicken's body having the opportunity to retain more nitrogen.

4. Conclusion

The level of use of fermented shrimp waste extract has an influence on the metabolizable energy value, nitrogen-corrected metabolizable energy, and nitrogen retention value in laying hens. The use of fermented shrimp waste extract is optimal at a rate of 1-2%, in the ration produces the highest metabolizable energy value and nitrogen-corrected metabolizable energy, while the highest retention value is shown in the ration with an addition rate of 1% in laying hens.

Suggestion

Fermented shrimp waste extract can be applied to the ration of laying hens, and it is recommended to use as much as 1-2%, in order to increase metabolizable energy value and nitrogen retention.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans' subjects by any of the authors.

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