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Research Paper

Probiotics Potential *Bacillus Amyloliquefaciens* in Various Counterweight Protein and Energy on the Performance Pullet Ration Chicken Laying

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***ABSTRACT**: This study aims to determine the effect of giving 3% probiotic B. amyloliquefaciens at various balancesprotein and energy differences in the pullet ration of laying hens. This study used 150 pullet laying hens aged 12 weeks for 6 weeks. This research method is an experimental method using Completely Randomized Design (CRD), with 10 treatments where the protein and energy balances are as follows: treatment A (15%: 2600), B (13%: 2300), C (14%: 2300), D (15%:2300), E (13%:2400), F (14%:2400), G (15%:2400), H (13%:2500), I (14%:2500), J (15%:2500) with 3 replicates. Parameters measured were ration consumption, body weight growth, and ration conversion. The results showed the addition of 3% probiotic B. amyloliquefaciens in drinking water at various balances Different protein and energy in the pullet ration of laying hens can reduce ration consumption, increase body weight gain, and streamline ration conversion. Based on the results of this study, it can be concluded that the addition of 3% probiotic B. amyloliquefaciens in drinking water can reduce the need for crude protein and metabolic energy in the ration, as seen from the undisturbed performance of laying hens pullets.*
***KEYWORDS:** Probiotics, Body Weight, Feed Consumption, Feed Conversion*

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I. INTRODUCTION

Pullets are laying hens that are reared at the age of 12-18 weeks in the grower phase. The maintenance phase of laying hens is divided into 3 phases, namely the starter phase (1 day - 6 weeks), the grower phase (age 6 - 18 weeks), and the layer/laying phase (aged 18 weeks - rejected) [2]. The achievement of body weight in accordance with the growth chart in the grower phase is one of the main indicators in achieving optimal egg production when in the production phase (layer). One of the most important things in this grower phase is the provision of quality feed. Quality feed is relatively more expensive, so it is necessary to manipulate nutrients to maximize nutrient supply, optimize feed costs and maximize production. One way to manipulate nutrients is to add feed additives in the form of probiotics.

Probiotics are non-pathogenic microorganisms that function to regulate the balance of microbes in the digestive tract through amechanism *competitive exclusion* which is starting to be widely used as a feed additive in livestock. Probiotics work by inhibiting the growth of nuisance organisms in the digestive system. According to [4], intestinal microbial balance will be achieved if beneficial microorganisms can suppress harmful microorganisms. This is because harmful pathogenic microbes are pushed out of the digestive tract ecosystem by normal digestive tract microbes or beneficial microbes. [14] stated that the provision of probiotics can maintain the balance of the composition of microorganisms in the digestive system of livestock, which increases the digestibility of feed ingredients and maintains the health of livestock. Probiotics can also increase the efficiency of the use of rations. [10], stated that the use of probiotics up to 3% in layer period layer chicken rations reduced consumption and feed conversion. [5], stated that the ration treatment with 3% local probiotics and 3% commercial probiotics showed a significant difference in the consumption and conversion of rations. One of the probiotics that can be used is *B. amyloliquefaciens*.

B. amyloliquefaciens is a bacterium isolated from the forest litter of Pesisir Selatan Regency, West Sumatra which has gram-positive characteristics, rod-shaped, produces elliptical endospores, clear zone on 27.85 mm CMC medium and cellulase activity of enzymes Cx and C1 on medium. high fiber (23.57%) were

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0.488 and 1,200 U/ml [21]. *B. amyloliquefacienscellulosic* isand can degrade crude fiber because it produces extracellular enzymes cellulase and hemicellulase [21]. According to [23], the administration of probiotic *B. amyloliquefaciens* through drinking water as much as 0.2%
in 6-week-old pitalah ducks reduces ration consumption, increases ration efficiency by more than 15%, increases total colony of *Bacillus sp* in the small intestine, and lowers intestinal pH. fine. This shows that *B. amyloliquefaciens* can increase the availability of protein and energy in the digestive tract so that the use of feed becomes efficient. *B. amyloliquefaciens* has been used in determining the protein and energy balance in layer-phase laying hens. According to [1], the administration of *B. amyloliquefaciens* to laying hens in the production phase (layer) with a protein:energy balance of 15%:2500 kcal can be used as the composition of the ration for laying hens. The best balance of protein and energy for pullets is at 15.5% protein composition and energy is 2700 kcal/kg [6], while the protein and energy balance for layer-phase laying hens is 16.50% protein and 2700 kcal/kg energy [6].

The right balance of protein and energy feed is an effort to find an efficient ration. The use of better probiotics for the performance of laying hens during the pullet period needs to be a concern, in addition to ration efficiency as well as to reduce feed costs which have always been an obstacle for farmers. Collaboration between the preparation of energy and protein balance with probiotic *B. amyloliquefacienspullets is* forexpected to reduce the use of protein and energy itself, so that the use of feed is more efficient. So it is expected that the administration of probiotic *B. amyloliquefaciens* can reduce protein and energy requirements in the ration, and does not interfere with the pullet performance of laying hens.

II. MATERIALS AND METHODS

Effect of giving 3 % probiotic *B. amyloliquefaciens* at various balancesprotein and energy differences in the pullet ration of laying hens. This study was conducted for 6 weeks to determine the effect of giving 3% probiotic *B. amyloliquefaciens* at various balancesdifferent protein and energy in the pullet ration of laying hens (12-18 weeks old) produced by PT. Japfa Multi Breeder.

Experimental Design The

Study used an experimental method using a completely randomized design with 10 treatments and 3 replications. Where the treatments are as follows:
treatment A (15% PK : EM 2600), B (13% PK : EM 2300), C (PK 14% : EM 2300), D (15% PK : EM 2300), E (PK 13 % : EM 2400), F (PK 14% : EM 2400), G (15% PK : EM 2400), H (PK 13% : EM 2500), I (PK 14% : EM 2500), J (PK 15 % : EM 2500) with 3 replicates.

Cages and Equipment The

Cage used is a battery cage measuring 35 cm wide × 45 cm long × 60 cm high, which is equipped with a place to eat and drink. The lighting of the cage uses a 60 watt incandescent lamp that is turned on at night.

Ration Treatment The

Ration consisted of concentrate 122 (PT. Charoen Pokphand Indonesia), corn, rice bran, mineral B12 (PT. Eka Farma). Probiotic *B. amyloliquefaciens was* given 3% via drinking water at 12 weeks of age. The content of food substances (%) and metabolic energy (kcal/kg) of the ingredients of the ration can be seen in Table 1. The composition of the treatment ration (%) and the content of food substances and metabolic energy of the combination of the treatment ration are presented in Table 2 and Table 3.

Table 1. The Content Of Food Substances (%) and Metabolic Energy (Kcal/Kg) of the Ingredients for the Research Rations.

Feed Ingredients	PK (%)	LK (%)	SK (%)	Ca (%)	P (%)	ME (Kcal/kg)
Corn[a]	8.25	3.9	3.23	0.03	0.42	3290
Bran[a]	10.55	11.35	13.82	0.11	1.2	1620
Concentrate 122[a]	29.78	3.75	7.67	3.89	0.91	2210
Mineral B12[a]	0	0	0	47.58	12.87	0

Source: a. Laboratory of Non-Ruminant Nutrition, Faculty of Animal Husbandry, Andalas University 2020

Table 2. Composition of Treatment Rations (%) Treated Feed

ingredients Feed Ingredients (%)	Treatment									
	A	B	C	D	E	F	G	H	I	J
Corn	50	36	34	33	41	40	38	47	46	44
Bran	19	44	42	38	39	35	32	33	29	26
Concentrate 122	30	19	23	28	19	24	29	19	24	29
Minerals B12	1	1	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100	100	100

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Probiotics	0	2	2	2	2	2	2	2	2	2

Table 3. Nutrient Content and Metabolic Energy of Treatment Ration

No	Treatment	PK (%)	LK (%)	SK (%)	Ca (%)	P (%)	EM(kcal)
1	A	15.06	5.23	6.54	1.68	0.84	2615.80
2	B	13.27	7.11	8.70	1.27	0.98	2317.10
3	C	14.09	6.96	8.67	1.43	0.98	2307.30
4	D	15.07	6.65	8.47	1.62	0.98	2320.10
5	E	13.16	6.74	8.17	1.27	0.94	2400.60
6	F	14.14	6.43	7.97	1.46	0.94	2413.40
7	G	15.15	6.20	7.87	1.65	0.94	2409.50
8	H	13:02	6.29	7.54	1.27	0.90	2500.80
9	I	14.00	5.99	7.33	1.46	0.89	2513.60
10	J	15.01	5.75	7.24	1.65	0.89	2509.70

Information: Obtained from Table 1 and Table 2.

Data Analysis The data

Obtained in using analysis of variance (ANOVA) according to the design used. The results of the analysis of diversity gave a real or very significant effect, then the Duncan Multiple Range Test (DMRT) was carried out to determine the differences between treatments [18].

$$Yij = +a+Ɛij$$

Information:
- Yij = Observation results on treatment i and repetition j
- a = Treatment ration
- i = Treatment (1, 2, 3, ..., 10)
- j = Deuteronomy (1,2, 3)
- Mean = value
- ij = Effect of residual (random) j on treatment i.

Parameters measured are as follows:

1. Consumption of Ration

Consumption research ration consumption is calculated by adding up consumption per week. Consumption of rations per week using the following formula.

Consumption of ration = Ration given – Ration remaining at the end of week

2. Body Weight Gain

Body weight gain is calculated based on the current week's body weight minus last week's body weight.

Body Weight Gain = body weight at the end of the week - body weight at the beginning of the previous week.

3. Conversion of Ration

Conversion of ration is the amount of ration consumed by livestock to produce one kg of body weight.

Conversion of Ration = Consumption (g)/Body Weight Gain (g)

Data Analysis

Parameters were statistically analyzed using diversity analysis according to the pattern of Completely Randomized Design (CRD) 10 treatments with 3 replications to determine the effect of treatment. Significant differences between treatments will be further tested with DMRT (Duncan's Multiple Range Test).

Time and Place of Experiment

This research was conducted at the Farm Unit and Non-Ruminant Laboratory, Faculty of Animal Science, Andalas University, Padang, Indonesia.

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III. RESULTS AND DISCUSSION

Ration Consumption

Table 4. Average Feed Consumption (Grams).

Treatment of	Feed Consumption (grams)	
	Average	SD
A Probiotics 0% (PK 15%, ME 2600 kcal)	3239.73^{ab}	18.67
B (PK 13%, ME 2300 kcal)	3247.67^{bc}	3.91
C (PK 14%, ME 2300 kcal)	3254.73^{c}	18:14
D (PK 15%, ME 2300 kcal)	3244.73^{abc}	16.62
E (PK 13%, ME 2400 kcal)	3246.20^{bc}	19:47
F (PK 14%, ME 2400 kcal)	3251.87^{bc}	8.30
G (15% PK, 2400 kcal ME)	3248.73^{bc}	10.24
H (13% PK, 2500 kcal ME)	3255.93^{c}	9.25
I (14% PK, 2500 kcal ME)	3232.13^{a}	2.30
J (15% PK, 2500 ME kcal)	3238.07^{ab}	13.74

Note: Different superscript letters in the same column show significant differences (P<0.05)
PK = Crude Protein
ME = Metabolic Energy
SD = Standard Deviation

Results of variance indicate that the addition of 3% probiotic *Bacillus amyloliquefaciens* has an effect significantly different (P<0.05) on feed consumption. DMRT test results showed that treatment A (0% probiotic) was not significantly different (P>0.05) compared to treatment D (PK 15%, ME 2300 kcal), I (PK 14%, ME 2500 kcal) and J (PK 15 %, ME 2500 kcal), but significantly different (P<0.05) from other treatments.

Consumption of the same ration between treatment A (0% probiotic with 15% PK, ME 2600 kcal) which had a higher energy and protein balance compared to treatment D (PK 15%, ME 2300 kcal), I (PK 14%, ME 2500 kcal) and J (PK 15%, ME 2500 kcal) which had a lower energy and protein balance but still showed the same ration consumption, this was due to the addition of 3% probiotic *Bacillus amyloliquefaciens* in drinking water thereby increasing protein and energy efficiency in the ration. According to [21], that B. *amyloliquefaciens* as a probiotic in the digestive tract produces enzymes -amylase, -acetolactate decarboxylase, -glucanase, maltogenic amylase, urease, protease, xylanase, chitinase, phytase, cellulase, hemicellulase, and lipase.

Probiotics can improve the digestive tract and increase feed digestibility, namely by suppressing pathogenic bacteria in the digestive tract so as to support the development of beneficial bacteria that help the absorption of food substances (Kompiang, 2002). Intestinal surface area to absorb nutrients was wider in chickens that receivedprobiotics *Bacillus* spcompared to those that did not receive probiotics [17]. Probiotics can change the movement of mucin and microbial populations in the small intestine of chickens, so that their presence can improve intestinal function and health, improve the composition of microflora in the cecum, and increase the absorption of nutrients [11].

Added by [21], that B. *amyloliquefaciens* can be used as probiotics because these bacteria meet the requirements needed as probiotics, including these bacteria produce heat-resistant endospores, have the ability to degrade xylan and carbohydrates, grow well at 40ºC and pH 6 , resistant to pasteurization and able to grow in high concentrations of salt solution (10%) [21]. B. *amyloliquefaciens* can survive in the small intestine of laying hens for 32 days with the number of colonies 18x10-7 CFU/gram of fresh small intestine, reducing 0.9% of ration consumption [12].

Low feed consumption in treatment B (PK 13%, ME 2300 kcal), C (PK 14%, ME 2300 kcal), E (PK 13%, ME 2400 kcal), F (PK 13%, ME 2400 kcal), G (15% PK, 2400 kcal ME) and H (13% PK, 2500 kcal ME), this is because the energy and protein balance in the ration is much different, so the ability of B. *amyloliquefaciens* as a probiotic is no longer able to improve the digestive tract and increase digestibility. feed. According to [3], Chickens have the ability to adjust the need for energy consumption in feed. This causes rations that have a high energy content to have lower ration consumption, because the energy needs of chickens have been met.

Weight Gain

Table 5. Average Body Weight Gain (Grams)

Treatment	Weight	
	Average	SD
A Probiotics 0%, (PK 15%, ME 2600 kcal)	396.33^{a}	12.17
B (PK 13%, ME 2300 kcal)	316.87^{c}	13.82
C (PK 14%, ME 2300 kcal)	323.93^{c}	11.04
D (PK 15%, ME 2300 kcal)	333.27^{c}	4.10
E (PK 13%, ME 2400 kcal)	350.87^{b}	11.92
F (PK 14%, ME 2400 kcal))	396.20^{a}	5.25

G (PK 15%, ME 2400 kcal)	402.13[a]	4.91
H (PK 13%, ME 2500 kcal)	364.80[b]	11.61
I (PK 14%, ME 2500 kcal)	412.93[a]	6.70
J (15% PK, ME 2500 kcal)	405.13[a]	8.60

Note: Different superscript letters in the same column show significant differences (P<0.05)
PK = Crude Protein
ME = Metabolic Energy
SD = Standard Deviation The

Results showed that the addition of 3% probiotic *Bacillus amyloliquefaciens had* an effect on significantly different (P<0.05) on body weight gain. The results of the DMRT test showed that treatment I (PK 14%, ME 2500 kcal) was not significantly different (P>0.05) compared to treatment A 0% probiotics, (15% PK, 2600 kcal ME), F (14% PK, 2400 ME). kcal), G (15% PK, 2400 kcal ME), and J (15% PK, 2500 kcal ME), but significantly different (P<0.05) from other treatments.

The same body weight gain between treatment A (0% probiotic with 15% PK, ME 2600 kcal) which had a higher energy and protein balance compared to treatment F (PK 14%, ME 2400 kcal), G (15% PK, ME 2400 kcal), I (14% PK, 2500 kcal ME) and J (15% PK, 2500 kcal ME) which have a lower energy and protein balance, because feed consumption is also the same so that growth is also the same, the increase in body weight gain is influenced by consumption feed because the nutrients, especially protein and energy that will be used for development, are contained in the feed consumed by the livestock. According to [16], protein in feed that is consumed efficiently can increase body weight gain. According to [22], that the ration must contain nutrients in sufficient and balanced condition, so that it can support maximum growth.

According to [19], that ration consumption is mainly influenced by body weight, performance, cage temperature, hair loss conditions, food texture, energy level, and energy balance. According to [15], stated that the balance between protein and energy in the ration affects the amount of ration consumption. According to [7], chickens consume rations to meet their energy needs. If the energy needs are not met, the chicken will continue to eat. Conversely, if the energy in the ration is high, then the chicken will reduce its consumption. Furthermore, [20] stated, if the protein content in the ration is sufficient and balanced, it will have the same effect on the consumption of the ration.

The use of 3% probiotic *Bacillus amyloliquefaciens* can increase feed efficiency, especially the use of protein and energy, this is because the probiotic *Bacillus amyloliquefaciens* can produce enzymes in the digestive tract that are useful for poultry. According to [21], that B. *amyloliquefaciens* as a probiotic in the digestive tract produces enzymes -amylase, -acetolactate decarboxylase, -glucanase, maltogenic amylase, urease, protease, xylanase, chitinase, phytase, cellulase, hemicellulase, and lipase.

In this study, the selected protein and energy balance based on body weight gain was treatment I (PK 14%, ME 2500 kcal). According to [8], which states that the protein of laying hens in the grower period aged 12-18 weeks is 15%.

Feed Conversion

Table 6. Mean Ration Conversion

Treatment	Feed Conversion	
	Average	SD
A Probiotic 0%, (PK 15%, ME 2600 kcal)	8.18[a]	0.29
B (PK 13%, ME 2300 kcal)	10.26[d]	0.44
C (PK 14%, ME 2300 kcal)	10.06[cd]	0.35
D (15% PK, 2300 kcal ME)	9.74[c]	0.17
E (13% PK, 2400 kcal ME)	9.26[b]	0.32
F (14% PK, 2400 kcal ME)	8.21[a]	0.13
G (15% PK) , ME 2400 kcal)	8.08[a]	0.12
H (PK 13%, ME 2500 kcal)	8.93[b]	0.32
I (PK 14%, ME 2500 kcal)	7.83[a]	0.13
J (PK 15%, ME 2500 kcal)	7.99[a]	0.14

Note: Letter Different superscripts in the same column showed significantly different (P<0.05)
PK = Crude Protein
ME = Metabolic Energy
SD = Standard Deviation The

Results of the analysis of variance showed that the addition of 3% probiotic *Bacillus amyloliquefaciens* had a significant effect (P<0.05) on the feed conversion. DMRT test results showed that treatment A (0% probiotic with 15% PK, ME 2600 kcal) was not significantly different (P>0.05) compared to treatment F (PK 14%, ME 2400 kcal), G (PK 15%, ME 2400 kcal), I (PK 14%, ME 2500 kcal) and J (PK 15%, ME 2500 kcal), but significantly different (P<0.05) with other treatments.

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The same ration conversion between treatment A (0% probiotic with 15% PK, ME 2600 kcal) which had a higher protein and energy balance compared to treatment F (PK 14%, ME 2400 kcal), G (15% PK, ME 2400 kcal), I (14% PK, 2500 kcal ME) and J (15% PK, 2500 kcal ME) which had a lower energy and protein balance, this was due to the same ration consumption and body weight gain in each treatment. This is in accordance with the opinion of [13] that ration conversion is a comparison of the amount of ration consumption in one week with the increase in body weight of chickens achieved that week.

The balance of energy and protein which was low compared to treatment A (0% probiotic with 15% PK, ME 2600 kcal) could increase the efficiency of its use with the addition of 3% probiotic *Bacillus amyloliquefaciens as* seen from the conversion of the treatment ration. This is because probiotics can digest feed ingredients in the intestine and increase the efficiency of the use of rations. [14], stated that the provision of probiotics can maintain the balance of the composition of microorganisms in the digestive system of livestock, which increases the digestibility of feed ingredients and maintains the health of livestock. Probiotics can also increase the efficiency of the use of rations. This study is similar to the statement of [10] and [5], which stated that the use of probiotics up to 3% in the ration showed a significant difference in the consumption and conversion of the ration.

IV. CONCLUSION

Based on the results of this study, it can be concluded that the administration of 3% probiotic *B. amyloliquefaciens* in drinking water with a crude protein balance of 14% and metabolic energy of 2500 kcal/kg can reduce protein and energy requirements in the ration, seen from the undisturbed performance (consumption, increase in energy consumption). body weight and ration conversion) pullet laying hens.

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