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Provision of Lactic Acid as Acidifier in Probiotics-Containing Ration on Protein Performance of Sentul Chicken

(Pemberian Asam Laktat sebagai Acidifier pada Pakan Probiotik terhadap Kinerja Protein Ayam Sentul)

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ABSTRACT. The aim of this research was to study the addition of an acidifier in feed containing probiotics on protein performance in Sentul chickens. The material used was 60 female Sentul chickens aged 6 months, kept for 2 months in 20 units of battery cages, and used a completely randomized design (CRD) with 4 treatments of 5 replications, therefore there were 20 experimental units with 3 head / replications. The variables measured included protein performance (protein consumption, liver protein percentage, and meat protein percentage and meat protein mass). Treatment: R0: basal feed; R1: basal feed + probiotic with lactic acid acidifier 0.5%; R2: basal feed + probiotic with lactic acid acidifier 1.0%; R3: basal feed + probiotic with lactic acid acidifier 1.5%. Data analysis with analysis of variance (ANOVA) using IBM SPSS. The results of the analysis of variance showed that lactic acid as an acidifier in feed containing probiotics had a significant effect (P <0.05) on protein consumption, liver protein percentage, meat protein percentage but had no significant effect (P> 0.05) on meat protein mass. Based on the results of the study, it can be concluded that the addition of a 0.5% acidifier in probiotics containing ration can be used as additional feed for Sentul chickens because it is efficient in optimizing protein performance.

Keywords: acidifier, probiotics, protein performance, Sentul chicken

ABSTRAK. Tujuan penelitian adalah untuk mengkaji penambahan *acidifier* dalam pakan yang mengandung probiotik terhadap kinerja protein pada ayam Sentul. Materi yang digunakan adalah ayam Sentul betina umur 6 bulan sebanyak 60 ekor yang dipelihara selama 2 bulan pada 20 unit kandang baterai, dan menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan 5 ulangan oleh sebab itu terdapat 20 unit percobaan dengan 3 ekor / ulangan. Variabel yang diukur meliputi kinerja protein (konsumsi protein, persentase protein hati, persentase protein daging dan massa protein daging). Perlakuan: R0: pakan basal; R1: pakan basal + probiotik dengan *acidifier* asam laktat 0,5%; R2: pakan basal + probiotik dengan *acidifier* asam laktat 1,0%; R3: pakan basal + probiotik dengan *acidifier* asam laktat 1,5%. Analisis data dengan analisis variansi (ANOVA) menggunakan IBM SPSS. Hasil analisis variansi menunjukkan bahwa penggunaan asam laktat sebagai *acidifier* pada pakan yang mengandung probiotik berpengaruh nyata (P<0,05) terhadap konsumsi protein, persentase protein hati dan persentase protein daging tetapi berpengaruh tidak nyata (P>0,05) terhadap massa protein daging. Berdasarkan hasil penelitian dapat disimpulkan bahwa penambahan *acidifier* dengan taraf 0.5% dalam pakan yang mengandung probiotik dapat mengoptimalkan kinerja protein ayam Sentul.

Kata kunci: *Acidifier*, ayam Sentul, kinerja protein, probiotik

INTRODUCTION[1]

Sentul chicken is a local chicken in Ciamis Regency, West Java Province. Apart from producing eggs, many Sentul chickens are raised for their eggs and meat. Sentul chicken has the advantage of relatively fast growth and high egg production (Hidayat and Sopiyana, 2010). Iriyanti *et al*. (2019) stated that the genetic potential of Sentul chickens as a producer of high native chicken eggs needs to be supported by high-quality feed nutrient content. Productivity can be optimal when poultry are given good-quality feed. The high quality feed can increase feed consumption, especially protein consumption.

Protein is needed by poultry because protein will support optimal poultry productivity. One of the efforts to increase the productivity and endurance of Sentul chickens can be done through the provision of feed additives, such as probiotics. Probiotics-containing lactic acid bacteria (LAB) increase the number of beneficial microbial populations and inhibit the development of harmful microbes in the digestive tract. The lactic acid bacteria growth can be optimized with the addition of an acidifier. Lactic acid can be used as a feed additive source. Acidifier is a feed additive that functions to control microflora in the digestive tract. Acidifiers as organic acids added to feed or drinking water can be used to improve livestock digestibility (Pratama *et al*., 2019). The

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lactic acid content in chicken feed can increase the productivity and health of chickens. The study purpose was to assess the addition of lactic acid as an acidifier in the feed containing probiotics on the protein performance of Sentul chicken.

MATERIALS AND METHOD

The research material used was 60 female Sentul chickens aged six months, which were kept for two months. The ingredients of the diet were corn, rice bran, probiotic bran, fishmeal, soybean meal, palm oil, $CaCO_3$, premix, L-Lysin HCl, methionine, and lactic acid as an acidifier. The ration was prepared with iso-calories and iso-protein. The protein and metabolizable energy contents were 18.39% and 2,853.60 kcal/kg respectively. The composition of the ration and the nutrient content of the ration is presented in Table 1 and Table 2.

Table 1. The composition of the ration

No.	Ration Ingredients	Composition (%)
1	Corn	40
2	Rice bran	18
3	Probiotic Bran	18
4	Soybean meal	14
5	Fish meal	5
6	Palm oil	1
7	Calcium carbonate ($CaCO_3$)	2
8	Premix	1
9	Lysine	0.6
10	Methionine	0.4
	Total	100

Table 2. Nutrient Content of Ration

No	Nutrient	Unit	Content
1.	Protein	%	18.39
2.	Energy	kcal/kg	2,853.60
3.	Crude fiber	%	5.05
4.	Crude fat	%	5.92
5.	Calcium	%	2.44
6.	Phosphorus	%	1,344
7.	Lysine	%	1,1451
8.	Methionine	%	0.6654

Source: analysis conducted at the Laboratory of Nutrition and Animal Feed Science, Faculty of Animal Husbandry, Jenderal Sudirman University (2020).

The research used was a completely randomized design. There were four treatments, namely R0: Basal Ration (BS); R1: BS + Probiotic with Acidifier / eubiotic lactic acid 0.5%; R2: BS + Probiotic with Acidifier / eubiotic lactic acid 1.0%, R3: BS + Probiotic with Acidifier / eubiotic lactic acid 1.5%. Each treatment was repeated five times to obtain 20 experimental units. The research was conducted for two months at the Experimental Farm and Animal Nutrition and Food Science Laboratory, Faculty of Animal Husbandry, Jenderal Soedirman University. Purwokerto.

The research design used was a completely randomized design. There were 4 treatments and 5 replications so that 20 experimental units were obtained, namely:

R0: basal ration (control)
R1: R0 + Probiotic with lactic acid acidifier 0.5% from the dry matter feed
R2: R0 + Probiotic with lactic acid acidifier 1.0% from the dry matter feed
R3: R0 + Probiotic with lactic acid acidifier 1.5% from the dry matter feed

Work of Sequence

Cage Preparation

Preparation of the cage includes washing the cage along with feed and drinking places, liming, disinfectant spraying, mixing and rice hucks, and putting chickens into each cage. The cage numbering is done randomly.

Isolation of Probiotic

Isolation of probiotics was carried out with isolated probiotic bacteria from the digestive tract of chickens and ducks by taking 1 gram of intestinal content and then diluting up to 104 with sterile distilled water. The diluted intestinal content was then plated using the pour plate method on MRSB (deMann Rogosa Sharp Broth) media. Then, incubated batch culture for 2x24 hours. Observations were made to grow LAB (Lactic Acid Bacteria)

Probiotic Bran Manufacturing (Iriyanti dan Suhermiyati, 2015)

Probiotic bran was made by mixing bran with isolates from chickens and ducks' intestines. Rice bran is weighed for 3 kg and then mixed with 1-liter aqua dest into the container. The next step is to wrap rice bran that has been mixed with water using plastics. The bran is put into an autoclave for sterilization. The sterile bran was then mixed with probiotics made from the intestinal content of chickens and ducks. The bran that had been mixed with the isolates was then wrapped again and incubated for seven days.

Making of Ration

Making of ration includes mixing the basal feed that consists of corn, rice bran, probiotic bran

soybean meal, fishmeal oil palm, premix, $CaCO_3$, methionine, and lysine. Then, the feed is mixed with lactic acid according to the level of treatment. The treatment ration were arranged in iso-protein and iso-calorie.

Raising of Sentul Chicken

Sentul chicken raising is done for two months namely from chicken aged 24 weeks until 32 weeks. Feeding is done two times specifically in the day and evening. Provision of drinking water ad libitum. Temperature and humidity were checked and recorded in the morning, afternoon, and evening.

Protein Performance Measurement

Protein Consumption: Protein consumption is the average amount of protein consumed by chickens during rearing. Protein consumption is expressed in grams, calculated by the formula according to Tillman (1991):

Protein consumption = feed consumption (g) × percentage of crude protein ration (%)

Meat Protein Mass: meat protein mass is an indicator of success in the utilization of feed protein into body tissues. Meat protein mass is expressed using the formula according to Suthama (2003):

Mass of meat protein = percentage of meat protein (%) × weight of meat (g)

Meat and Liver Protein Levels: samples were selected after weighing the weights. Samples taken for each treatment have an average weight between the lightest and heaviest weights. Preparation begins with slaughtering the chicken, then the breast and thigh meat and liver samples will be analyzed for protein. Measuring protein content by proximate analysis according to AOAC (2005) includes three stages, namely destruction, distillation, and titration.

Destruction

Weigh the sample 0.1 gram (X), put it in the Kjeldahl flask, add a little catalyst and 1.5 ml of concentrated H_2SO_4 do the destruction in the acid cupboard, first on low heat, and let it change color from brown to clear green, cool to room temperature.

Distillation

The distillation device is installed, starting from the stove and cooler, enter the results of the destruction in the top funnel of the distillation apparatus and add 10 ml of 40% NaOH, the funnel and flask are cleaned with distilled water after the funnel cover valve is closed, the distillation container is prepared with a 125 ml Erlenmeyer filled with 10 ml of 2-3% boric acid, the distillation was terminated until the volume in the Erlenmeyer reservoir reached a volume of 60 ml.

Titration

The results of the distillation are then titrated using 0.1 N HCl, before the titration add a little (1-2 drops) of mixed indicator. At the end of the titration, when the color changes from green to purple, record the volume of the titrant.

$$\%\ \text{Crude Protein} = \frac{\text{ml titran x N HCl x } 0.014007 \text{ x } 6{,}25}{\textit{Sample weigh}}\ x\ 100\%$$

6.25 = Conversion factor for protein content of ingredients

0.014007 = 1 ml of solution N contains 0.01407 g nitrogen and every 1 g protein contains 16%

RESULT AND DISCUSSION

The results of the study showed that the average protein consumption, percentage of liver, and meat protein and meat protein mass in Sentul chicken blood that received the addition of an acidifier in feed containing probiotics were presented in Table 3.

Table 3. The Average protein performance in Sentul chickens

Parameter	Perlakuan			
	R_0	R_1	R_2	R_3
Feed Consumption	89.17±2.46	84.48 ±4.40	87.50 ±4.76	97.24±2.61
Protein consumption** (g/ekor/hari)	16.39±0.47^{a}	15.62±0.76^{a}	16.16±0.89^{a}	17.89±0.48^{b}
Liver protein percentage** (%)	18.37±0.31^{a}	19.23±0.64^{b}	19.23±0.71^{b}	18.60±0.81^{a}
Meat protein percentage** (%)	19.33±1.16^{a}	20.79±1.05^{b}	19.53±1.38^{a}	19.44±0.72^{a}
Meat protein mass* (g/ekor)	33.23±1.96^{ab}	40.92±1.48^{b}	40.75±1.07^{b}	31.15±1.76^{a}

Description: * significant effect; **very significant effect;
R_0 (basal ration/control); R_1 (basal ration + lactic acid 0.5%); R_2 (basal ration + lactic acid 1%); R_3 (basal ration + lactic acid 1.5%).
^{a,b} Different superscripts in the same column indicate there is a difference in treatment (P<0.05).

Protein Consumption

The average protein consumption from the research results ranged from 15.62-17.98 g / head / day or a mean of 16.52 g / head /day. Differences in results between treatments are very likely to occur considering that, there are differences in treatments and the feed given to chickens which can affect the consumption patterns of the poultry themselves. The analysis of variance showed that the treatment had a very significant effect (P <0.01) on the consumption of crude protein in Sentul chickens.

The results of further tests using the Honestly Significant Difference test (HSD) showed that the treatment of R3 was significantly different from other treatments. The R3 treatment with the addition of 1.5% acidifier showed the highest protein consumption results. Giving an acidifier with a level of 1.5% from feed increases protein consumption. It is related to the level of palatability of poultry to feed containing 1.5% acidifier. The feed-in treatment R3 has a taste and aroma, which is preferred by chickens, thereby increasing palatability so that feed consumption also increases.

The increase in feed consumption in poultry affects the increase in protein consumption in Sentul chickens that receive an additional 1.5% acidifier. In line with the opinion of Hidayah *et al*. (2017) that the fragrant aroma of acidity can increase palatability and animal feed consumption. Palatability is largely determined by the texture, aroma, and color of the feed, which are physical and chemical factors of the feed.

According to Roth and Kischgessener (2003), the ideal use of an acidifier in feed is 0.2–1%. However, in this study, an acidifier was used to reach a level of 1.5%, and it can still be tolerated because protein consumption tends to increase to a level of 1.5%. Harmayanda *et al*. (2016) stated that the crude protein content in feed for laying hens is at least 16%. The protein content of the ration used in this study was 18.39%, so the addition of an acidifier could optimize the protein content of the ration and was very helpful in increasing the nutritional intake of Sentul chickens.

Liver Protein Percentage

According to Siswandy *et al*. (2020), the liver is an endocrine and exocrine gland in the chicken body. Al-Samawy *et al*. (2016) stated that the liver is also one of the largest glands in the body, which can detoxify metabolites and catalyze biosynthetic and biodegradative processes. The results of this study showed that the percentage of liver protein in Sentul chickens ranged from 18.37-19.23% or with an average of 18.86% (Table 3). The analysis of variance showed that the treatment had a very significant effect (P <0.01) on the percentage of liver protein in Sentul chickens. The Honest Real Difference test (HSD) shows that there is a significant difference between treatment R0, R3 and treatment R1, R2. The results of the R0 and R3 treatments had adjacent liver protein percentages, as well as R1 and R2 treatments, which had adjacent liver protein percentages. Table 3 shows that the liver protein percentage between R1 and R2 was the highest at 19.23±0.64 and 19.23±0.71 respectively.

Table 3 shows that the percentage of the liver was highest in the R1 and R2 treatments, but the protein consumption was low. It means that protein consumption of 15.62-16.16 g/head/day can give a good effect on a more optimal percentage of liver protein. It means that the use of an acidifier in feed containing probiotics for Sentul chickens as much as 0.5 to 1% is effective in increasing the percentage of liver protein. It means that the work of the liver is not too heavy to neutralize excess protein that cannot be metabolized. As a result, the protein that is absorbed in the liver becomes more optimal. While the use of a 1.5% acidifier in feed is considered less effective in increasing the percentage of liver protein because the results are not different from feeding without the addition of an acidifier. It is likely because the intestinal atmosphere is too acidic, resulting in a decrease in the protease enzyme activity. The reduced activity of these enzymes causes protein digestibility to decrease so that the percentage of liver protein also decreases. So that the addition of an acidifier in feed containing probiotics is not recommended to exceed the 1% level because the results are not significantly different from the treatment without an acidifier.

The liver has a complex function, including the metabolism of carbohydrates, fats, proteins, and iron (Budiman *et al*., 2015). The Indonesian Ministry of Health (1996) stated that the chemical composition of chicken liver varies, but in general, it has a high nutritional value with 19.70% protein content, 3.2% fat, and 69.70% water. It is in line with the study result that liver protein in Sentul chickens ranged from 18.37-19.23%.

Meat Protein Percentage

The results showed that meat protein in this study ranged from 19.33-20.79% or with an average of 19.77% (Table 3). The highest meat protein percentage was found in the R1 treatment with the addition of an acidifier of 0.5%. The results of the study are in accordance with the opinion of Aberle *et al*. (2001) that the protein content of meat in chicken ranges from 16-22%. The results of the analysis of variance showed that the treatment had a very significant effect ($P<0.01$) on the percentage of meat protein. The addition of an acidifier in the feed containing probiotics affects the meat protein percentage in Sentul chickens. It is because the acidifier functions to optimize the digestive process in the chicken digestive tract, especially the protein digestion process. Optimal digestibility of protein causes the supply of protein in the body of poultry to be optimal, including the protein supply to poultry products, one of which is meat. Giving an acidifier in feed containing probiotics also increases the immunity of Sentul chickens, causing the protein in the chicken's body to not be widely used for tissue repair and antibody formation so that the protein deposited in the meat becomes higher.

The optimal meat protein percentage is supported by the protein content in the ration and absorption in the digestive tract. The use of an acidifier of 0.5% in feed containing probiotics has been able to increase the performance of the percentage of meat protein in Sentul chicken. Radhiyani *et al*. (2017) stated that the acidifier functions in the protein digestion process to optimize protein absorption by livestock. The results of further tests using the Honestly Significant Difference (HSD) showed that the R1 treatment was significantly different from the other three treatments. Treatment R1 is a treatment with 0.5% acidifier in feed containing probiotics. Probiotics function to manipulate microorganisms in the chicken intestines so that they can help increase the absorption of feed protein. Acidifier in this case helps optimize the work of probiotics in the digestive tract of chickens, which causes optimal absorption of feed nutrients, especially protein. The addition of organic acids (acidifier) to drinking water or broiler feed is proven to be able to increase absorption by increasing the function of digestive enzymes so that it affects digestion and absorption, especially fiber and protein (Atapattu and Nellisgaswatta, 2005; Abdel-Fattah *et al*., 2008). Khodijah *et al*. (2012) stated that the use of protein is very important for chickens; therefore, additional feed is given to improve the efficiency of the use of rations.

Meat Protein Mass

The results are presented in Table 3, which shows that the average meat protein mass is around 32.15-40.92 g/head or with an average of 36.51 g/head. The results of the analysis of variance showed that the treatment had a significant effect ($P<0.05$) on the meat protein mass. It is because the meat protein mass is related to the percentage of meat protein. The higher meat protein percentage will be followed by an increase in the mass of meat protein. The results of further tests using the Honestly Significant Difference (HSD) showed that the R1 treatment was significantly different from the other treatments and was the treatment with the highest mass yield of meat protein. It is related to the percentage of meat protein that the percentage of protein in the meat will also determine the meat protein mass. Meat protein mass is used as an indicator of the good or not protein deposition in chicken meat. Jamilah *et al*. (2013) stated that protein intake has an important role in the protein deposition process. High protein deposition in meat indicates that the chicken's body absorbs more feed protein.

Table 3 shows that the treatment with the highest mass of meat protein was the R1 treatment, namely with the addition of an acidifier of 0.5%. It means that the addition of a 0.5% acidifier has been able to optimize protein deposition in chicken meat. In addition, this study used a probiotic-based ration. According to Astuti *et al*. (2015), probiotics will affect the physiological function of the intestine by modulating the microflora of the digestive tract and the mucosal immune system of the digestive tract to improve the health of the digestive tract, especially the small intestine as an organ for absorption of ration nutrients. Lactic Acid Bacteria (LAB) in the acidifier will work to synthesize lactic acid to lower the pH of the intestine as a result, an acidic atmosphere will arise in the intestine, which causes the growth of pathogenic bacteria to be inhibited, and nutrient absorption runs optimally. The addition of 0.5% acidifier in feed containing probiotics has a positive effect on bacteria in the small intestine and does not interfere with the performance of enzymes in the digestive tract.

CONCLUSION

The results showed that the addition of an acidifier was able to improve the protein performance of female Sentul chickens. The addition of a 0.5% acidifier level in feed containing probiotics efficiently increases the protein consumption, percentage of liver protein, and meat protein percentage but does not give a significant effect on the protein mass of Sentul chicken.

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