Research Article

Effect of Probiotic Supplementation *Lactococcus plantarum* and *Pediococcus pentasaceus* with Purple Sweet Potato Media on Performance and Egg Quality of Laying Hens

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Abstract | Probiotics are live microorganisms that can improve the health of humans or livestock by balancing the microflora in the digestive tract when consumed in sufficient quantities. Purple sweet potato has high effectiveness against LAB growth, namely 10,771 (log10 cfu/g) with 85.34% resistance be used as a medium for LAB growth. This study aims to determine the effect of probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* using a purple sweet potato carrier on laying hen's performance and egg quality. This study used 210 layers of medium-type laying hens, Strain Isa Brown, 38 weeks old. The method used was an experiment with a completely randomized design (CRD) with seven treatments and three replications. The observed variables were ration consumption, ration conversion, egg mass production, hen day production (HDP), egg weight, shell thickness, and egg cholesterol. The results showed that supplementation of probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* using a purple sweet potato carrier at a dose of 2 grams could affect daily egg production, egg mass and reduce the value of ration conversion, shell thickness and egg cholesterol, but doses of 1 gram and 2 grams did not affect ration consumption and egg weight. It was concluded that the administration of the best LAB probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* with a dose of 2 grams.

Keywords | Egg quality, *Lactococcus plantarum*, *Pediococcus pentasaceus*, Production performance, LAB probiotics

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INTRODUCTION

Eggs are animal foods that have high nutritional value. In addition, eggs are the most frequently consumed food ingredient by the community because the price is relatively affordable and easy to obtain (Hasym, 2016). Eggs widely consumed by the community are eggs from layer chickens (Pribadi et al., 2015). Indonesia's laying hens business sector has experienced many fluctuations and obstacles, including the unstable price of chicken eggs, while the price of feed tends to increase. So that the income received by farmers is not proportional to the price of feed provided, where the cost of feed reaches a value of 70% of production costs, therefore, it is necessary to give probiotics to increase the efficiency of the feed given.

Probiotics are live microorganisms that can improve the health of humans or livestock by balancing the microflora in the digestive tract when consumed in sufficient quantities. The bacteria that acts as a probiotic is Lactic Acid Bacteria (LAB). LAB are gram-positive, rod-shaped, or spherical bacteria that convert carbohydrates into lactic acid (Korhenen, 2010; Aritonang et al., 2017). Essential characteristics that must be considered in selecting probiotic strains are safe or harmless, beneficial to the host, persist in the digestive tract and adhere to the intestine (Saarela et al., 2000), can lower cholesterol levels, and improve egg quality (Lei et al., 2013; Dwyana et al., 2019; Anggraeni et al., 2020).

Lactococcus plantarum and *Pediococcus pentasaceus* are LABs originating from blondo and dadiah Sijunjung (Husmaini et al., 2011; Purwati, 2011). Giving *Lactococcus plantarum* to laying hens as much as 3 ml (3.9 x 108 CFU/ml) can reduce egg yolk cholesterol levels up to 53.6%. Meanwhile, giving *Pediococcus pentasaceus* at a dose of 3 ml (3.81 x 10^7 CFU/g) improved egg quality. This quality improvement, among others, increases the egg Haugh unit but does not affect the thickness of the shell and egg yolk color in Pitalah ducks (Yunensi, 2011) and can reduce the cholesterol content of eggs.

Giving LAB to animal feed is generally given orally/fresh, but this is less efficient when applied to chickens reared in large numbers. Therefore, another alternative is needed to give probiotics to poultry to make it more effective and efficient, namely by giving a carrier medium. The results of research by Husmaini et al. (2013) found that the best types of solid carriers for LAB from Virgin Coconut Oil (VCO) waste were purple sweet potato and corn, where the amount of LAB that was able to grow on purple sweet potato media was 10,771 ± 0.247 (log 10 cfu/g). The use of purple sweet potatoes as carriers of LAB, has a high resistance, which is 85.34%, relatively the same as corn, which is 85.47%. This best carrier is due to the complex carbohydrate content in purple sweet potatoes and the content of anthocyanins, which function as antioxidants and contain oligosaccharides, which are the main components of prebiotics (Husmaini et al., 2013).

Oligosaccharide compounds (short-chain polysaccharides) consist of raffinose, stachyose, and verbascose. They cannot be digested by digestive enzymes, thus suppressing the growth of harmful bacteria in the intestines. Therefore, the administration of LAB probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* with a carrier medium in purple sweet potato is expected to increase feed efficiency and positively impact production performance and egg performance quality produced.

MATERIAL AND METHODS

Material

This study used 210 layers of medium-type laying hens, Strain Isa Brown, at the layer phase of 38 weeks. The type of probiotic supplementation used in this study were *Lactococcus plantarum* and *Pediococcus pentasaceus* probiotics in solid form using a purple sweet potato carrier (Husmaini et al., 2020). The cages used in this study were individual battery cages measuring 40 cm x 30 cm x 30 cm as many as 210 units.

Research Ration

The ration given is a self-mixed ration consisting of corn meal, concentrate, fine bran, coconut cake, bone meal, and top mix. The content of food substances and metabolic energy of the ration are presented in Table 1. In contrast the composition of the ration material (%) and the nutritional content and metabolic energy are presented in Table 2.

Method

This research was conducted using an experimental method with a completely randomized design (CRD) with seven treatments and three replications. With details of treatment as follows:
A = Control (ration without probiotics)
B = *Lactococcus plantarum* dose of 1 gram
C = *Lactococcus plantarum* dose of 2 grams
D = *Pediococcus pentasaceus* dose of 1 gram
E = *Pediococcus pentasaceus* dose of 2 gram
F = *Lactococcus plantarum* + *Pediococcus pentasaceus* dose of 1 gram
G = *Lactococcus plantarum* + *Pediococcus pentasaceus* dose of 2 grams
Probiotic administration is carried out once a week in the morning. In order for all probiotics to be consumed by chickens, probiotics are mixed with 25% of the ration of the day's needs. After that the ration is again given *ad-libitum*

Data analysis

The data obtained were analyzed using analysis of variance according to the design used, namely Completely Randomized Design; with Microsoft Excel and Minitab14 applications.

RESULT AND DISCUSSION

Observations on the performance (feed consumption, feed conversion, Egg Mass Productions, and Hen Day Egg Production) of laying hens can be seen in Table 3, is presented in two observations, namely period I (38-41weeks) and period II (42-45weeks). The treatment of probiotics LABs Lactococcus plantarum and Pediococcus pentasaceus did not affect the feed consumption (p>0.05). Feed consumption is closely related to the energy level of the ration. Chicken are able to regulate their energy consumption well (Wahyu, 2004). In this research, all treatments used the same ration formulation, so it did not affect the amount of feed consumption.

The feed conversion was measured by comparing the

Table 1: Proximate analysis and metabolic energy of feedstuff

Ingredients	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ca (%)	P (%)	Metabolic Energy (kcal/kg)
Corn Milled	7.51	1.05	3.73	0.18	0.25	3264
Concentrate	34.80	6.78	3.45	3.17	0.16	3020
Rice Bran	11.00	6.29	12.50	0.10	0.21	1630
Coconut Meal	6.54	26.4	11.85	0.27	0.15	5056
Bone Meal	0	0	0	18.66	0.22	0
Top Mix	0	0	0	5.38	1.14	0

Table 2: Composition of Feedstuffs (%), Nutrient Content and Energy Metabolism of Diet

Ingredients	Composition (%)	Crude protein (%)	Crude Fat (%)	Crude Fiber (%)	Ca (%)	P (%)	ME (kcal/kg)
Corn Milled	51.02	3.83	0.54	1.90	0.09	0.13	1665.29
Concentrate	25.51	8.88	1.73	0.88	0.81	0.04	770.40
Rice Bran	15.31	1.68	0.96	1.91	0.02	0.03	249.55
Coconut Meal	2.55	0.17	0.68	0.30	0.01	0	128.93
Bone Meal	5.10	0	0	0	0.95	0.01	0
Top Mix	0.51	0	0	0	0	0	0
Total (%)	100	14.56	3.90	5.00	1.87	0.22	2814.18

Note: Calculation based on Table 1.

Table 3: Effect of probiotics against production performance of laying hen

Treatment	Ration Consumption		Conversion Ration		Egg Mass Productions		Hen Day Egg Production	
	Period I (38–41 weeks)	Period II (42-45 weeks)	Period I	Period II	Period I	Period II	Period I (%)	Period I (%)
A	108.20	109.91	2.90^{a}	2.67^{a}	37.93^{c}	41.31^{b}	64.40^{c}	64.40^{c}
B	104.68	106.58	2.51^{abc}	2.40^{b}	42.80^{ab}	44.82^{b}	71.90^{ab}	71.90^{ab}
C	109.62	111.30	2.46^{bc}	2.39^{b}	43.92^{ab}	46.77^{a}	74.88^{ab}	74.88^{ab}
D	110.21	111.65	2.82^{ab}	2.49^{b}	39.48^{bc}	45.15^{ab}	67.98^{bc}	67.98^{bc}
E	107.87	107.67	2.48^{bc}	2.39^{b}	43.83^{ab}	45.17^{ab}	75.36^{a}	75.36^{a}
F	111.15	111.13	2.84^{ab}	2.51^{b}	39.51^{bc}	44.62^{b}	67.74^{bc}	67.74^{bc}
G	107.28	109.41	2.37^{c}	2.48^{b}	45.01^{a}	44.21^{b}	77.10^{a}	77.10^{a}

Note : Means within different superscripts $^{(a,b,c)}$ in the same column are significantly effect ($p<0.05$)

Table 4: Effect of probiotics against egg quality of laying hens

Treatment	Egg Weight		Eggshell Thickness		Egg Cholesterol	
	Period I (g/egg)	Period II (g/egg)	Period I (mm)	Period II (mm)	Period I (mg/dl)	Period II (mg/dl)
A	58.34	59.47	0.37^{b}	0.38^{b}	$209,83^{a}$	191.80^{a}
B	59.02	59.33	0.40^{ab}	0.41^{ab}	201.97^{a}	155.60^{ab}
C	58.60	59.75	0.42^{a}	0.42^{ab}	98.53^{b}	85.98^{c}
D	58.11	59.21	0.39^{ab}	0.41^{ab}	170.27^{a}	140.48^{b}
E	57.86	58.78	0.43^{a}	0.43^{a}	93.87^{b}	88.75^{c}
F	58.25	58.90	0.40^{ab}	0.41^{ab}	182.67^{a}	118.55^{bc}
G	57.60	58.61	0.42^{a}	0.43^{a}	93.67^{b}	77.93^{c}

Note : Different superscripts $^{(a,b,c)}$ in the same column show significant effect ($p<0.05$)

amount of ration consumed with the weight of the eggs produced (Fenita et al., 2010b) while the egg mass production was the percentage of daily production multiplied by the average egg weight. The results showed a significant effect ($p<0.05$) on the ration conversion and egg mass obtained. Feed conversion has a close relationship with egg mass production. Giving probiotics causes egg mass production to increase even though the ration consumption is not different, so that egg conversion becomes smaller. Increasing egg mass was due to the favorable atmosphere in the digestive tract for the absorption of rations, resulting in a change in microflora balance in the intestine, which was characterized by an increased egg mass. The administration of probiotics using purple sweet potato carriers causes the administration of probiotics to be more effective against laying hens; this is due to the high content of antioxidants and beta-carotene and oligosaccharides that can be used for the growth of probiotic microbes (Husmaini et al., 2013).

Hen Day Production (HDP) is an egg production in a group of laying hens based on the percentage of egg production by the number of laying hens during the study (Huda et al., 2019). The HDP obtained showed a significant effect ($p<0.05$), where the best LAB probiotics were *Lactococcus plantarum* and *Pediococcus pentasaceus* at a dose of 2 grams. According to El-Hack et al. (2020), probiotics affected to improve intestinal villus height. Increasing the villus height and architecture of the crypts in the gastrointestinal tract allows for the improvement of nutrient digestion and absorption. The administration of probiotics could be balanced in the microbial population in the digestive tract. The population of beneficial microbes is more than the population of harmful microbes so that the digestive process runs well and there is an increase in egg production. Probiotics grown on purple sweet potato caused the administration of probiotics to laying hens to be more effective, causing antimicrobial activity, enzymatic activity, and energy metabolism of feed in the digestive tract to increase, which was followed by an increase in daily egg production. According to Huda et al. (2019) giving probiotics have positive affects the absorption of nutrients that affect the number of ova produced so that the performance of the reproductive organs is maximized and production is also optimal.

Observation of the effect of using LAB probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* on the quality of broiler eggs (egg weight, shell thickness, and egg cholesterol) in periods I and II can be seen in Table 4. Egg weight in laying hens was not statistically significant ($p>0.05$). The average egg weight of laying hens period I and II ranged between 57.60-59.02g/egg and 58.61-59.75g/egg. There was an increase in the average egg weight from period I to period II. Probiotics can affect the balance of microflora in the intestine so that digestion and absorption of food substances become better. The ability of *Lactococcus plantarum* bacteria to produce lactic acid as the main product in lowering the pH and creating an acidic atmosphere in the intestines. Acidic conditions in the intestine cause the growth of pathogenic microbes to be inhibited. In addition, lactic acid bacteria increase the absorption of food in the intestine due to reduced adhesion of pathogenic microbes. Budiansyah (2004); Zurmiati et al. (2014) stated that the mechanism of action of probiotics in poultry includes competition for receptors for attachment or adhesion to the epithelium in the digestive tract and competition for nutrients for growth.

Eggshell thickness statistically showed a significant effect ($p<0.05$) on the treatment in periods I and II. The average thickness of the shells of laying hens period I and II ranged from 0.37 to 0.43 mm and 0.38 to 0.43 mm. The administration of probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* could increase the thickness of the shells compared to those without probiotics. Egg shell thickness increased with probiotics at 2 g compared to 1 g. Probiotics Lactic Acid Bacteria *Lactococcus plantarum* and *Pediococcus pentasaceus* can affect the thickness of eggshells because microbes in the intestine can stimulate the performance of the oviduct as a place for making shells to produce a strong shell. According to Fuller (2001), giving probiotics causes absorption of minerals such as calcium and P to be good, so this study resulted in good shell thickness. Nahashon et al. (1994); Vidyani et al. (2015) explained that the decrease in pH on the intestines of laying hens caused by probiotics would increase calcium absorption phosphorus from the feed in the small intestine and mineralization of bones and eggshells. The formation of egg shells is needed for calcium and potassium and other substances. Purple sweet potatoes generally also contain carbohydrates, sugar, vitamins A, B, C, calcium and potassium. According to Oguntunji and Alabi, (2010) egg shells are influenced by genetic traits, calcium in feed, hormones, environment and maintenance management. Purple sweet potatoes are high in antioxidants and beta-carotene. In addition, purple sweet potato also contains oligosaccharides that can be used by microbes for growing. The results of Panda et al. (2003) also found that the administration of probiotics (probiolac at the level of 100 mg/kg ration) could improve egg production, shell weight, and eggshell thickness.

The probiotic treatment had a significant effect ($p<0.05$) on the cholesterol of eggs in both period I and II observation. Based on the results of the DMRT test, it was shown that the use of 2 g of Lactic Acid Bacteria *Lactococcus plantarum* and *Pediococcus pentasaceus* probiotics could reduce the cholesterol levels of laying hen eggs. The decrease in cholesterol levels is caused by changes in the balance of microflora in the intestines, thereby increasing the population of Lactic Acid Bacteria in the digestive tract. Lactic acid bacteria produce bile salt hydrolase (BSH), this enzyme will break down bile acids into bile acids and deconjugate them in the form of free cholic-acid which is absorbed by the small intestine excreted through feces.

Winarsih (2005) also explained that LAB is firmly attached to the intestinal wall and prevents the colonization of pathogenic microbes in the intestine. The opportunity for *Escherecia coli* and *Salmonella* sp. to stick to the intestine is much reduced and will be excreted with feces. The increasing number of good microbes causes inhibition of the enzyme Hydroxi Methyl Glutaryil - CoA reductase (HMG-CoA reductase), which plays a role in the formation of mevalonate in the cholesterol synthesis process so that cholesterol does not form. Voet et al. (1999) also explained that the decrease in cholesterol in poultry occurs because the metabolites produced by microbes compete with HMG CoA to bind to the HMG-CoA reductase enzyme. Thus, the decrease in cholesterol is due to the ability of probiotics to deconjugate bile salts (Liong et al., 2005). The mechanism is that BSH hydrolyzes or breaks the C-24 N-Acyl amide bond formed between bile acids and amino acids in conjugated bile salts to produce deconjugated bile salts and glycine/taurine. Deconjugated bile salts have low solubility, are more hydrophobic and are passively absorbed directly by the intestinal mucosa back to the liver through the bloodstream (Astuti and Ana, 2010).

BSH also plays a role in water molecules between glycine or taurine with cholic acid which produces unconjugated bile salts. The formed cholic acid is less absorbed by the small intestine than conjugated bile salts, thus bile acids that return to the liver during enterohepatic circulation are reduced and excreted in the feces (Fajrina et al., 2014). So the deconjugated bile acids pass out through the feces and cause more cholesterol needed for synthesizing bile salts again, thereby reducing cholesterol levels in the body and also reducing cholesterol transfer in chicken ovaries so that the cholesterol contained in laying hen eggs decreases. Mahdavi et al. (2005) and Husmaini et al. (2013) also explained that giving probiotics to chickens improved the quality of chicken eggs, including a decrease in cholesterol and triglyceride levels in chicken eggs. Based on the research, it can be concluded that the best dose of LABs probiotics *Lactococcus plantarum* and *Pediococcus pentasaceus* is 2 grams. This dose can increase daily egg production, egg mass, and shell thickness. On the other hand, It decreased feed conversion and cholesterol levels and reduced egg yolks but did not affect feed consumption and egg weight.

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CONFLICT INTEREST

The authors have declared no conflict of interest.

AUTHOR'S CONTRIBUTION

Husmaini, Sabrina, Firda Arlina, Linda Suhartati and Yozella Martilova contributed to conducting research, data processing and writing this manuscript.

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