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The Effect of Probiotic Derived from Kumpai Minyak (*Hymenachne Amplexicaulis*) Silage on Performance and Egg Quality Characteristics of Pegagan Ducks

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ABSTRACT

The study aimed to determine the effect of probiotics derived from an isolate of Kumpai Minyak grass silage on performance and the physical egg quality of Pegagan ducks. The study was conducted in 16 weeks, from May to September 2020. The sample size was 400 female Pegagan ducks aged five months. The treatments included basal diet (Control) and base diet plus 0.2% (P2), 0.4% (P4), 0.6% (P6), and 0.8% (P8) probiotic silage of Kumpai Minyak grass. The observed variables were performance (egg production, egg weight, feed consumption, and feed conversion ratio) and physical quality (albumen index, albumen weight, yolk weight, and Haugh unit). Observation data on probiotic treatment 0.8% (P8) established a significant effect on egg weight, compared to other treatments. Moreover, P8 probiotic treatment could significantly affect daily egg production and feed conversion ratio, compared to P2 and P4 probiotic treatments. Different results were found in the observations on feed consumption, where the overall treatment diet indicated significant results, compared to the control treatment. Specifically, several variables showed a significant effect, namely albumen index, albumen weight, egg yolk weight, and Haugh unit. Each observed variable value increased along with increasing probiotic treatment levels. However, egg index, egg yolk index, shell weight, and thickness were inversely related to the other variables investigated in this study. The P8 probiotic treatment could increase digestibility and absorption of feed nutrients due to inhibition of pathogenic bacteria and optimization of the digestive tract. The probiotics at the level of 0.8% produced from the Kumpai grass silage process can be used as a growth promoter for laying ducks to replace commercial antibiotic products.

Keywords: Albumen, Antibiotic, Growth promoter, Isolate, Probiotic, Silage

INTRODUCTION

Pegagan ducks are local ducks originating from Indonesia and are widely available in the southern part of Sumatra. Pegagan ducks are dual-purpose avians meaning that they can produce meat and eggs. Sari et al. (2011) reported that the average weight of Pegagan duck eggs was over 70 g, and this value was relatively high, compared to other local duck eggs. However, the poor maintenance system left the large potential of Pegagan duck unfulfilled leading to a high risk of disease, insufficient nutrient needs, and consequently, low productivity (Sari et al., 2014). One of the efforts to answer this problem is to provide feed additives, such as antibiotics, in animal feed to improve performance and protect poultry production to be more resilient in the face of various invading diseases (Raphael et al., 2017; Amine et al., 2020). However, the use of

antibiotics as feed additives has been banned because of the residues they produce (Costa et al., 2018; Sweeney et al., 2018). Antibiotics are generally used to maintain the digestive tract condition by controlling the balance of microflora in ducks' digestive tract. Several experiments have been carried out to overcome or find alternative solutions to replace these antibiotics, including probiotics and organic acid compounds (Sandi et al., 2019; El-Kholy et al., 2020).

Probiotics are live microorganisms that are added to animal feed to increase the balance of the intestinal microflora in order to increase nutrient absorption and increase livestock performance (Chen and Yu, 2020). Until now, many studies have been carried out to find effective and efficient probiotics against poultry in general, such as the use of isolated microorganisms to

produce the expected probiotics (Al-Khalaifah, 2018). Furthermore, the use of probiotics from silage isolates has become a new trend among researchers to find probiotics or derivative compounds produced to benefit the world of animal husbandry (Sari et al., 2019; Sandi et al., 2021).

Indonesia is a tropical country whose territory consists of various islands. It has multiple types of land, such as sup-optimal land (swamps), making Indonesia a country that has great potential in finding numerous kinds of probiotics that can be isolated from various types of green vegetation. The probiotics that are being developed and come from forages or plants in swamps are probiotics from Kumpai grass silage (*Hymenachne amplexicaulis*). The type of probiotic produced is a type of lactic acid bacteria. Swamp grass silage can be used as a probiotic because the lactic acid bacteria produced have characteristics such as gram-positive, non-spore, catalase-negative, non-motile, and not form spores (Sandi et al., 2018). (Jannah, 2017) reported that probiotic from copper Kumpai grass silage significantly affected the total lactic acid bacteria needed to accelerate the decrease in pH. The total lactic acid bacteria produced from the manufacture of probiotics was 8.24 (107 CFU / ml), and the resulting isolates had high resistance to acids, which could survive at pH 2.5 and pH 7. According to Fauziah et al. (2013), the use of probiotics containing 3.6 ml of lactic acid bacteria can work well in the digestive tract by increasing ration consumption. According to an *in vitro* study by Sandi et al. (2019), that Lactic acid bacteria (LAB) isolated from Kumpai grass silage as a probiotic showed resistance and can survive and thrive at different pH levels.

The use of probiotics both in feed and drinking water can help improve enzyme Activity. Based on Zhang et al. (2012) research, the addition of probiotics can increase egg production, which will affect the physical quality of the eggs. Based on this description, a study was conducted on the effect of providing probiotic Kumpai grass on the egg quality characteristics of Pegagan ducks.

MATERIALS AND METHODS

Ethical approval

An animal feeding experiment was conducted at the experimental station, Department of Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The ducks were cared for according to the Animal Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from Universitas Sriwijaya.

Study design

The study used a completely randomized design (CRD) with five probiotic treatments, which included a diet without probiotics (Con), diet + Probiotics 0.2% (P2), diet + Probiotics 0.4% (P4), diet + Probiotics 0.6% (P6), and diet + Probiotics 0.8% (P8). The feed used in the study was a formulation diet made from corn, rice bran, concentrate, meat and bone meal, premix, methionine, and lysine. Meanwhile, The used probiotics were collected from lactic acid bacteria isolated from copper Kumpai grass silage. Lactic acid bacteria isolates were cultured in MRSB (deMannRogosa Sharpe Agar in liquid/broth form) and then incubated for 48 hours. The bacterial culture was centrifuged at 3000 rpm for 15 minutes to obtain the substrate from the supernatant. The substrate was mixed with skim milk and 5% (w/w) maltodextrin. The next step is to spray dry at a temperature of 160-180°C to produce a dry powder product which can then be added to the diet according to the treatment (Bregni et al., 2000).

Management and sample collection

In the current study, the pegagan ducks used came from the Kotodaro village community farm, Tanjung Raja district, Ogan Ilir regency (OI), South Sumatra Province, Indonesia. As for the selected female ducks, they were already in the laying phase and had physical characteristics of blackish-brown fur color and shiny blue wings black. A total of 400 female Pegagan ducks aged five months were randomly assigned to 5 treatment groups, each consisting of 4 replications (20 ducks per replication, 80 ducks per treatment). For each replication, ducks were housed separately in cage size of 2000 m². In accordance with recommendations for good management of poultry raising, ducks were subjected to the same humidity, temperature, feeding regime, drinking water, and lighting (Cherry and Morris, 2008).

The study was conducted in 16 weeks, from May to September 2020. During the trial period, chickens were provided with feed and drinking water *ad-libitum*, while the compartment temperature measurement was ranged from 15 to 28°C. The basal diet used is presented in Table 1. In this experiment, the observed variables consisted of the observation of performance and egg quality. Observation of performance data included consumption, conversion, egg production, and egg weight. Meanwhile, egg quality analysis included Haugh units, egg size, albumen index, and egg yolk. At the beginning and end of the experiment, body weight was measured, which is then Based on the difference between the times, the weight gain is calculated. The feed consumed was chopped at a three-

day interval. Feed consumption was recorded at the beginning and end of the trial period, then calculated as gram/hen/day. The feed conversion ratio was calculated as kilograms of feed consumed per kilogram of egg produced.

Table 1. Nutrient composition of diets of Pegagan Ducks

Ingredients	Amount (g/kg)
Maize (Corn)	484
Rice bran	185
Meat Bone Meal	64
Konsentrat	245
Premix	10
Metionin	8
Lysin	4
Calculated energy and Chemical analysis	
Metabolisme Energy (MJ/kg)	2750.80
Dry matter, (%)	89.09
Crude Fiber, (%)	3.78
Ether Extract, (%)	7.36
Crude Protein, (%)	20.94
Calcium (%)	3.31
Phosphorus, (%)	1.08
Ash (%)	2.66

Furthermore, all eggs collected and weighed based on the treatment were then determined as egg weight. Based on these observations, egg production, egg weight, and daily egg yield were calculated. Egg quality was selected for three consecutive days at the 30-day trial and at the end of the test. A total of 20 eggs were randomly collected from each replication on the third and sixth day of the experiment. Each egg was weighed, and the shape index was calculated as a percentage according to the formula (egg length) / (egg width) with the instrument (shape index instrument, 75135/2, BV. Apparatenfabriek Van Doorm, De Bilt, Netherlands). Eggshell thickness was measured using a micrometer and the yolk color was determined using the Roche Yolk Fan. Haugh unit was calculated according to the formula of Nesheim et al. (1979):

$$\text{Unit Haugh (\%)} = 100 \times \log(H + 7.57 - 1.7W^{0.37})$$

where H is the albumen height, and W is the egg weight.

Data analysis

The data obtained were analyzed by variance analysis (ANOVA). If the treatment significantly affected the observed variables (p < 0.05), the analysis was

continued with Duncan New Multiple Range Test (DNMRT) test using the SPSS program (version 20).

RESULTS AND DISCUSSION

Performance

The results of the analysis can be seen in Table 2. Overall daily egg production, egg weight, feed consumption, and FCR showed significant results (p < 0.05). Observation data on P8 has established a considerable effect on egg weight, compared to other treatments (p < 0.05). The same results were also obtained for daily egg production and FCR, where there was a significant effect on P8 probiotic treatment, compared to P2 and P4 probiotic treatments (p < 0.05). However, it was not significantly different from the control treatment and P6 (p > 0.05). Regarding feed consumption, the overall treatment diet showed significant results, compared to the control treatment (p < 0.05), however, there were no significant differences among the probiotic treatments (p > 0.05).

Analysis of performance data, including egg weight and daily egg production, is often tested on laying chickens. The increase in egg weight followed by an increase in the level of treatment during the study could occur probably due to the high concentration of probiotic bacteria lactic acid in the Kumpai grass silage given, which led to optimal absorption of nutrients in the digestive tract. Furthermore, an increase in the value of daily egg production was also shown in treatment P8. This occurs presumably because of the close relationship between consumption value and the conversion of the treated diet. Consumption and feed conversion have an essential role in measuring livestock performance because the amount of consumption value can be used as a benchmark for determining nutrient intake obtained by livestock.

In contrast, the conversion was used as a benchmark to determine absorbed nutrients and was employed for livestock to meet their maintenance and production needs. Hajiaghapor et al. (2018) and Yu et al. (2020) reported that prebiotic or probiotic supplementation in the ration of laying hens could improve the health of the digestive system of these animals as evidenced by the high activity of lactic acid bacteria and an increase in the length and width of villi in the jejunum and ileum. In another study, Mikulski et al. (2020) reported that probiotics in rations with low and medium energy composition in laying poultry showing the probiotic supplementation on low-

energy rations led to an increase in consumption value and a decrease in conversion value, thus affecting the performance.

Based on the result of this study, strong suspicions were set against lactic acid bacteria in the form of *Lactobacillus plantarum* as the main factor causing the increase in Pegagan ducks' performance, which included egg weight, daily egg production, consumption, and feed conversion. Lactic acid bacteria is a type of bacteria that is widely used as a probiotic in livestock in general because of its ability to reduce or inhibit the growth of pathogenic bacteria, such as *Escherichia coli* in the digestive tract (Patterson and Burkholder, 2003; Khan and Naz, 2013; Al-Khalifa et al., 2019). These results correlate with previous studies that show that giving probiotics isolated from Kumpai grass silage tends to affect carcass weight gain, which is thought to be due to increasing nutrient absorption efficiency (Sari et al., 2019).

According to Sandi et al. (2018), the types of lactic acid bacterial strains in the Kumpai grass silage are *Lactobacillus plantarum* strains. Qiao et al. (2019) showed that *Lactobacillus plantarum* has the potential as a feed supplement in the laying hen industry because it has a good influence at the genus level on intestinal development digestibility of laying hens. *Lactobacillus plantarum* can produce lactic acid, which contains bacteriocin bioactive compounds in the digestive tract and have antibacterial activity so that they can kill or inhibit the growth of pathogenic bacteria in the digestive tract (Choe et al., 2012; Ahmed et al., 2014; Bali et al., 2016). However, Sjöfjan et al. (2020) reported that 0.8% *Lactobacillus plantarum* concentration did not show significant differences at concentrations of 0.2%, 0.4%, and 0.6% on egg weight but was significantly different from the control treatment.

Table 2. Effect of dietary treatments on performance of Pegagan Ducks

Variable	Treatment					SEM	p-value
	Con	P2	P4	P6	P8		
Egg weight (g)	56.96 ^a	59.09 ^b	62.32 ^c	64.30 ^d	68.36 ^c	0.279	< 0.05
Daily egg yield (g/hen/day)	56.96 ^{ab}	52.59 ^a	52.32 ^a	63.55 ^b	62.87 ^b	1.252	< 0.05
Feed consumption (g/hen/day)	367.51 ^a	385.68 ^b	400.06 ^b	399.03 ^b	394.79 ^b	2.274	< 0.05
Feed conversion ratio (g/g)	6.45 ^a	7.37 ^{ab}	7.78 ^b	6.28 ^a	6.35 ^a	0.159	< 0.05

Con: Diet without probiotics, P2: Diet + Probiotics 0.2%, P4: Diet + Probiotics 0.4%, P6: Diet + Probiotics 0.6%, P8: Diet + Probiotics 0.8%, SEM: Standard error means. ^{abc} Means in the same row without common letter are different at $p < 0.05$

Table 3. Effect of dietary treatments on the egg traits of Pegagan Ducks

Variable	Treatment					SEM	p-value
	Con	P2	P4	P6	P8		
Egg Shape Index (%)	77.09	80.20	78.03	77.46	79.85	0.545	0.30
Albumen Index (%)	0.063 ^a	0.088 ^b	0.090 ^b	0.085 ^b	0.098 ^b	0.002	< 0.05
Yolk Index (%)	0.338	0.370	0.393	0.420	0.408	0.018	0.64
Albumen Weight (%)	26.41 ^a	29.31 ^b	30.41 ^{bc}	30.48 ^{bc}	32.02 ^c	0.313	< 0.05
Yolk Weight (%)	21.31 ^a	21.53 ^a	23.81 ^{ab}	25.27 ^b	26.50 ^b	0.404	< 0.05
Eggshell Weight (g)	78.33	84.35	81.20	89.95	102.50	0.257	0.07
Eggshell Thickness (mm)	0.543	0.543	0.600	0.538	0.538	0.017	0.71
Haugh Units	61.31 ^a	71.36 ^b	73.56 ^b	73.88 ^b	73.75 ^b	0.906	< 0.05

Con: Diet without probiotics, P2: Diet + Probiotics 0.2%, P4: Diet + Probiotics 0.4%, P6: Diet + Probiotics 0.6%, P8: Diet + Probiotics 0.8%. SEM: Standard error means. ^{abc} Means in the same row without common letter are different at $p < 0.05$

Egg quality

The effect of probiotic-enriched feed on egg properties is given in Table 3. In particular, several variables show a significant effect, namely albumen index, albumen weight, yolk weight, and Haugh unit; the value of

each observed variable has increased along with increasing probiotic treatment levels. The best results were found in treatment P8, namely providing a diet with 0.8% probiotics for each variable. The provision of probiotics

did not affect these variables such as egg index, egg yolk index, shell weight, and thickness.

The high and low egg index, which includes the albumen index and the yolk index, is strongly influenced by the albumen and yolk weights. In this study, the observation of the albumen index showed that the probiotic treatment at each level was significantly different compared to the control. These results have a positive correlation with the increase in albumen weight in eggs treated with probiotics. However, different results were shown on the egg yolk index, which did not show a significant difference, although yolk weight showed an increase with increasing dose or level of probiotics in the feed. Furthermore, the increase in the observed variables carried out was thought to have a strong relationship with ducks' high-performance data shown in Table 2. Due to the high value of ¹³sumption and conversion of treatment rations, the high absorption of nutrients into the body of the livestock will affect the productivity of the eggs produced, including egg weight and egg quality parameters. Zhang et al. (2012) reported that probiotics in lactic acid bacteria could increase daily egg production, egg weight, and feed conversion value even though the resulting consumption values are not significantly different.

Furthermore, previous studies also revealed that probiotic supplementation had a significant effect on increasing egg production and egg quality (Zhang and Kim, 2013; Bidura et al., 2019; Mikulski et al., 2020). The egg index value, which is inversely proportional to the resulting yolk weight, is thought to be closely related to a decrease in fat and cholesterol content in eggs because of lactic acid probiotics (Li et al., 2011). However, Selim et al. (2020), in their report, stated that antioxidant compounds and bio-active compounds contained in feed could result in a high percentage of albumen and yolk weight in laying hens.

Table 3 shows that there is an increase in the Haugh unit value of eggs given probiotic treatment compared to control. Haugh unit value is generally used as an indicator of albumen in eggs. The high Haugh unit value is directly proportional to the increase in albumen weight. Besides, this increase strengthens the notion that developing lactic acid bacteria causes an increase in the digestive health system, resulting in increased nutrient absorption in the livestock body. Similar research results regarding the use of probiotics in livestock rations that affect Haugh units have been found in the last 10 years (Zhang and Kim, 2013; Bidura et al., 2019; Mikulski et al., 2020; Selim and Hussein, 2020).

8

CONCLUSION

Based on the current research results, it can be concluded that probiotics at the level of 0.8% produced from the Kumpai grass silage process can be used and contribute as a growth promoter for laying ducks to reduce using commercial antibiotic products. In further studies, it is recommended to test the combination of treatments with macro and micro minerals

DECLARATIONS

Competing interests

The authors declare no conflict of interest

Authors' contributions

All authors contributed to the design and implementation of the research, the analysis of the results, and the writing of the manuscript.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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