BUKTI KORESPODENSI
ARTIKEL JURNAL INTERNATIONAL BEREPUTASI

Judul Artikel	: The Effect of Probiotic Providing from Kumpai Minyak (Hymenache Amplexicaulis) Silage on Performance and The Eggs Physical Quality of Pegagan duck
Jurnal	: The Journal of the world poultry Research, 2022, Volume 12(1): 31- 37.
Penulis	: Sofia Sandi, Fitra Yosi, Eli Sahara, Asep Indra Munawar Ali, Nuni Gofar, Nur Muhamad

No	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit	11 Oktober 2021
2	Bukti Revisi editorial dan Konfirmasi proses review yang pertama.	18 Oktober 2021
3	Bukti konfirmasi review dan hasil review pertama	7 Desember 2021
4	Bukti konfirmasi Submit hasil Revisi Pertama	15 Desember 2021
5	Bukti konfirmasi Hasil review ke-dua	26 Desember 2021
6	Bukti konfirmasi Submit hasil revisi ke-dua	10 Januari 2022
7	Bukti konfirmasi Hasil review ke-tiga	20 Januari 2022
8	Bukti konfirmasi artikel accepted beserta invoice publikasi	30 Januari 2022
9	Bukti konfirmasi artikel published online	22 Maret 2022

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Tanggal: Senin, 11 Oktober 2021 pukul 15.00 WIB

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Manuscript Title: *	The Effect of Probiotic Providing from Kumpai Minyak (Hymenache Amplexicaulis) Silage on Performance and The Eggs Physical Quality of Pegagan duck
Manuscript Type: *	Research
Request Fast Peer-Review? *	Yes
Submission Type: *	New
Attach Document: *	jwpr.docx 43.45 KB · DOCX
Declaration Form	dokumen_64.pdf 383.82 KB · PDF

ABSTRACT:
(Do not omit from manuscript file.) *

This study aimed to determine the probiotic effect derived from an isolate of silage Kumpai Minyak grass on performance and the physical quality of pegagan ducks. The research was conducted in 16 weeks. The sample used was 400 female pegagan ducks at five months old. The treatments were diet based without treatment (Con) 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 weight). Observation data on probiotic treatment 0.8% (P8) has established a considerable effect on egg weight compared to other treatments ($P < 0.05$). The same results were also shown in daily egg production and feed conversion ratio, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment ($P < 0.05$). Different results were found in the observations on feed consumption, where the overall treatment diet showed significant results compared to the control treatment ($P < 0.05$). 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, other observations show that the variable egg index, egg yolk index, shell weight, and thickness are inversely related to the variables. The high value of probiotic treatment was due to its ability to inhibited pathogenic bacteria and optimize the digestive tract, thus increased digestibility and absorption of feed nutrients. The probiotics at the level of 0.8% produced from the kumpai grass silage process can be used as an antibiotic growth promoter (AGP) for laying duck to replace the role of commercial antibiotic products. v

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Sofia Sandi, Fitra Yosi, Eli Sahara, Asep I.M. Ali, Anggriawan N.T. Pratama, Nunik Gofar, Asmak, Muhamad N. Rofiq and Nur Muhamad
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Please:
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-Include authors' names and affiliations, ORCD, and Email address of the corresponding author in the revised word file.
-Indicate the corresponding author
-Add the Acknowledgements, Competing Interests, Ethics Committee Approval, and Authors' Contributions to the revised form (Separated titles).
-Add DOI number to the end of each reference. Example: DOI:https://doi.org/10.1016/j.livsci.2016.09.003
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Reviewers Guide

PART A: *Editorial Board*

Manuscript Number:	87
Title:	The effect of Probiotic Providing from Kumpai Minyak (Hymenache amplexicaulis) silage on Performance and The Eggs Physical Quality of Pagagan Duck.
Authors:	
E-Mail:	

PART B: *Reviewer Only*

SECTION 1: Comments per Section of Manuscript

General comment:	The title of this manuscript must be revised. Materials and Methods not easy to understand. But it is a good study.
Introduction:	Satisfactory
Methodology:	Some ambiguous statements were detected. Needs revision. Refer to my comments in the manuscript
Results:	Excellently done
Discussion:	Satisfactory. Please refer to my comments in the manuscript.
Bibliography/References:	The JWPR Author was not followed. ~~to the last~~
Others:	Authors are advised to revise the manuscript to improve its quality.
Decision:	

SECTION 2 - Please rate the following: *(1 = Excellent) (2 = Good) (3 = Fair) (4 = poor)*

Originality:	2
Contribution To The Field:	2
Technical Quality:	2
Clarity Of Presentation :	3
Depth Of Research:	2

SECTION 3 - Recommandation: (*Kindly Mark With An X*)

Accept as is	
Requires Minor Corrections:	
Requires Moderate Revision:	X
Requires Major Revision:	
Submit To Another Publication Such As:	
Reject On Grounds Of (Please Be Specific):	

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Please add any additional comments (Including comments/suggestions regarding online supplementary materials, if any):

Language Editor:
Scientific Editors and Reviewers:

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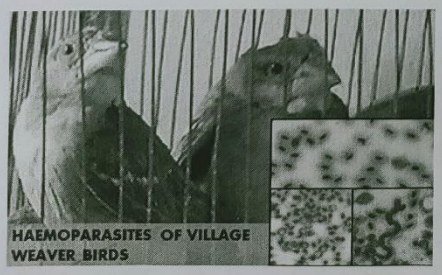

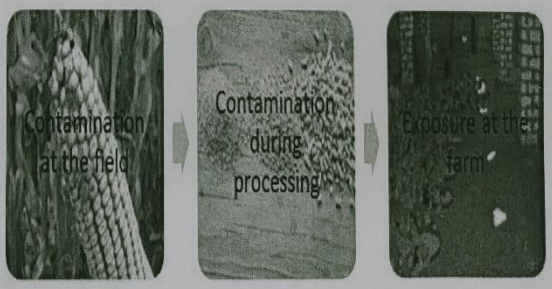

Original Article

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

Sofia Sandi[a*], Fitra Yosi[a], Eli Sahara[a], Asep I.M. Ali[a], Nuni Gofar[b], and Nur Muhamad[c]

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Abstract

The study aimed to determine the effect of probiotic derived from an isolate of silage Kumpai Minyak grass on performance and the physical egg quality of Pegagan ducks. The study was conducted in 16 weeks, from May to September (from when to when?). The sample size was 400 female Pegagan ducks aged five months. The treatments were diet without treatment (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) has established a significant effect on egg weight, compared to other treatments . The same results were also shown in daily egg production and feed conversion ratio, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment. Different results were found in the observations on feed consumption, where the overall treatment diet showed 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, other observations showed that egg index, egg yolk index, shell weight, and thickness are inversely related to the variables. The probiotic treatment at the level of 0.8% was due to its ability to inhibit pathogenic bacteria and optimize the digestive tract, thus increasing digestibility and absorption of feed nutrients. 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

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Pegagan ducks are local ducks originating from Indonesia and are widely available in the southern part of Sumatra. Pegagan ducks are dual-purpose meaning that they can produce meat and eggs. Sari et al. (2011) reported that the average weight of Pegagan duck eggs was over 70 grams, and this value was relatively high compared to other local duck eggs. However, the large potential of Pegagan duck is not supported by a good maintenance system, which is still traditional/extensive so that the productivity is still very low due to the high risk of disease and insufficient nutrient needs (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 ~~livestock~~ poultry production to be more resilient in the face of various invading diseases (Amine et al., 2020; Raphael et al., 2017). 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 (El-Kholy et al., 2020; Sandi et al., 2019).

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 probiotics 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. Similarly, Sandi et al. (2019) reported that Lactic acid bacteria (LAB) isolated from Kumpai grass silage as a probiotic and tested *in vitro* showed resistance implying ~~that it~~ and can survive and thrive at different pH levels.

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

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Materials and Methods

Ethical approval

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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 study used a completely randomized design (CRD) with five probiotic treatments, namely the basal diet without addition (Con) and the based diet added with 0.2% (P2), 0.4% (P4), 0.6% (P6), and 0.8% (P8) probiotics. Kumpai grass silage??~~ The feed used in the study was a formulation diet made from corn, rice bran, concentrate, meat and bone meal, premix, methionine, and lysine.

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Meanwhile, The probiotics used in this study 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 ~~The next step was to spray dry at 160-180°C. This process is that the powder product is ready to be added to the diet~~ (Bregni et al., 2000).

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Management and sample collection

In this study, the pegagan ducks used came from the Kotodaro village community farm, Tanjung Raja district, Ogan Ilir regency (OI), South Sumatra Province. As for the selected female ducks, they are already in the laying phase and have physical characteristics in the form of a 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

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The female ducks that do not produce the eggs infront of your visitors.

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housed separately in cage size of 2000 m^2. 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. 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 2 categories: 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. ~~At the beginning and end of the experiment, body weight was measured. Based on this value, the body weight gain was calculated.~~ The feed consumed was chopped with 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.

Furthermore, all eggs collected and weighed based on the treatment were then determined as egg weight ~~values.~~ Based on these observations, egg production, egg weight, and daily egg yield are calculated. Egg quality was selected for three consecutive days at the 30-day trial and the end of the test. Twenty 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. Apparatenfabreik 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, the analysis was continued with Duncan New Multiple Range Test (DNMRT) test using the SPSS program ver.20.~~Data obtained were analyzed using variance (ANOVA). If the treatment had a significant effect on the observed variables, the analysis was continued with the Duncan New Multiple Range Test (DNMRT). Further tests using the SPSS (version 20).~~

Results and Discussion

Performance

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Commented [JM36]: Confusing statement, please revise. A formula and then instrument?

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The results of the analysis that have been carried out 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 probiotic treatment 0.8% (P8) has established a considerable effect on egg weight compared to other treatments ($p < 0.05$). The same results were also shown in daily egg production and FCR, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment ($p < 0.05$). However, it was not significantly different compared with the control treatment and the probiotic 0.6% (P6) ($p < 0.05$). Different results were found in the observations on feed consumption, where the overall treatment diet showed significant results compared to the control treatment ($p < 0.05$) and did not differ significantly between probiotic treatments ($p > 0.05$).

Analysis of performance data, including egg weight and daily egg production, is often tested on laying birds. 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 is used as a benchmark to determine absorbed nutrients and is used by 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 ~~livestock~~ the performance.

In 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 E. coli in the digestive tract (Patterson and Burkholder, 2003; Khan and Naz, 2013; Al-Khalaifa 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).

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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, Sjofjan 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.

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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. Furthermore, the results were inversely proportional to the variable egg index, egg yolk index, shell weight, and thickness.

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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 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 consumption 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; Park et al., 2016; Bidura et al., 2019; Mikulski et al., 2020; Selim and Hussein, 2020).

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Conclusion

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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 as a growth promoter for laying ducks to reduce using commercial antibiotic products.

DECLARATIONS

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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.

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Table 1. Nutrient composition of diets of Pegagan Ducks

Ingredients	Amount (g/kg)
Maize (Corn)	484
Rice bran	185
Meat Bone Meal (MBM)	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

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

~~Item~~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[e]	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

Diet without probiotics (Con); diet + Probiotics 0.2% (P2); diet + Probiotics 0.4% (P4); diet + Probiotics 0.6% (P6);diet + Probiotics 0.8% (P8); Standart Error Means (SEM)

[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

~~Item~~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

Diet without probiotics (Con); diet + Probiotics 0.2% (P2); diet + Probiotics 0.4% (P4); diet + Probiotics 0.6% (P6);diet + Probiotics 0.8% (P8); Standart Error Means (SEM)

[abc] *Means in the same row without common letter are different at p <0.05*

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4. Bukti konfirmasi Submit hasil Revisi Pertama (15 Desember 2021)

On Wed, Dec 15, 2021 at 3:56 PM editor jwpr <editorjwpr@gmail.com> wrote:

Dear Author,

We received the revised article.

Best wishes

On Wed, Dec 15, 2021 at 3:55 AM Sofia Sandi <sofiasandi_nasir@yahoo.com> wrote:

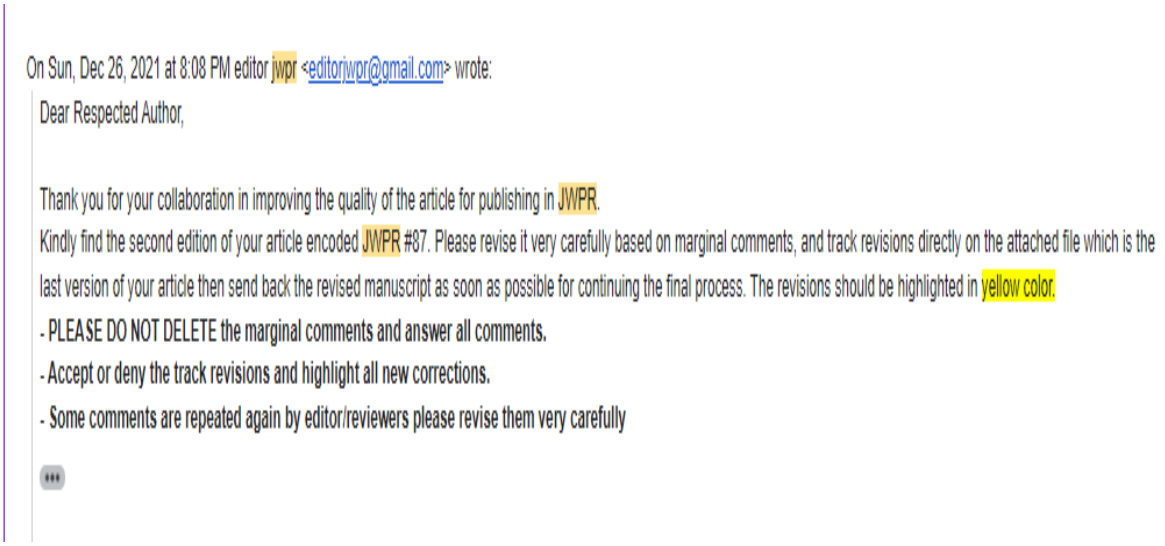
To our respected editors,

via this email we would like to send our article which is had been revised. thank you for the comments and suggestions that have been given. We really hope that the article that we have fixed can be published on JWPR

Best Regards,

Sofia Sandi

5. Bukti konfirmasi Hasil review kedua (26 Desember 2021)



On Sun, Dec 26, 2021 at 8:08 PM editor jwpr <editorjwpr@gmail.com> wrote:

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Kindly find the second edition of your article encoded JWPR #87. Please revise it very carefully based on marginal comments, and track revisions directly on the attached file which is the last version of your article then send back the revised manuscript as soon as possible for continuing the final process. The revisions should be highlighted in yellow color.

- PLEASE DO NOT DELETE the marginal comments and answer all comments.
- Accept or deny the track revisions and highlight all new corrections.
- Some comments are repeated again by editor/reviewers please revise them very carefully

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Original Article

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

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Abstract

The study aimed to determine the effect of probiotic derived from an isolate of silage Kumpai Minyak grass 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 were diet without treatment (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) has established a significant effect on egg weight, compared to other treatments . The same results were also shown in daily egg production and feed conversion ratio, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment. Different results were found in the observations on feed consumption, where the overall treatment diet showed 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, other observations showed that egg index, egg yolk index, shell weight, and thickness are inversely related to the variables. The probiotic treatment at the level of 0.8% was due to its ability to inhibit pathogenic bacteria and optimize the digestive tract, thus increasing digestibility and absorption of feed nutrients. 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

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Pegagan ducks are local ducks originating from Indonesia and are widely available in the southern part of Sumatra. Pegagan ducks are dual-purpose meaning that they can produce meat and eggs. Sari et al. (2011) reported that the average weight of Pegagan duck eggs was over 70 grams, and this value was relatively high compared to other local duck eggs. However, the large potential of Pegagan duck is not supported by a good maintenance system, which is still traditional/extensive so that the productivity is still very low due to the high risk of disease and insufficient nutrient needs (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 (Amine et al., 2020; Raphael et al., 2017). 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 (El-Kholy et al., 2020; Sandi et al., 2019).

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 probiotics 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. Similarly, Sandi et al. (2019) reported that Lactic acid bacteria (LAB) isolated from Kumpai grass silage as a probiotic and tested *in vitro* showed resistance implying 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 physical quality of the eggs. Based on this description, a study was conducted onthe 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 probiotics used in this study 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. As for the selected female ducks, they were already in the laying phase and had physical characteristics of a 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 m^2. 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 2021. 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

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Commented [D9]: What was the age of ducks?

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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 with 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.

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 are 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. Apparatenfabreik 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):

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

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 that have been carried out 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 probiotic treatment 0.8% (P8) has established a considerable effect on egg weight compared to other treatments ($p < 0.05$). The same results were also shown in daily egg production and FCR, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment ($p < 0.05$). However, it was not significantly different compared with the control treatment and the probiotic 0.6% (P6) ($p < 0.05$). Different results were found in the observations on feed consumption, where the overall treatment diet showed significant results compared to the control treatment ($p < 0.05$) and did not differ significantly between probiotic treatments ($p > 0.05$).

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Commented [ap16R15]: 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 are 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.

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Analysis of performance data, including egg weight and daily egg production, is often tested on laying birds. 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 is used as a benchmark to determine absorbed nutrients and is used by 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.

In 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 E. coli in the digestive tract (Patterson and Burkholder, 2003; Khan and Naz, 2013; Al-Khalaifa 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, Sjofjan 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.

Egg quality

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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. ~~Furthermore, the results were inversely proportional to the variable egg index, egg yolk index, shell weight, and thickness.~~

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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 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 consumption 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; Park et al., 2016; Bidura et al., 2019; Mikulski et al., 2020; Selim and Hussein, 2020).

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Conclusion

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Mention the contributionof your study to the field then make suggestion for future studies.

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.

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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

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Table 1. Nutrient composition of diets of Pegagan Ducks

Ingredients	Amount (g/kg)
Maize (Corn)	484
Rice bran	185
Meat Bone Meal (MBM)	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

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^{e}	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: Standart 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: Standart error means
^{abc} Means in the same row without common letter are different at p < 0.05

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6. Bukti Konfirmasi Submit hasil revisi ke-dua (10 Januari 2022)

---------- Pesan yang Diteruskan ----------
Dari: "editor jwpr" <editorjwpr@gmail.com>
Kepada: "Sofia Sandi" <sofiasandi_nasir@yahoo.com>
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Terkirim: Kam, 13 Jan 2022 pada 2:20
Judul: Re: Journal of World's Poultry Research-Review Results for Manuscript ID #87
Received with thanks.

On Wed, 12 Jan 2022, 19:51 Sofia Sandi, <sofiasandi_nasir@yahoo.com> wrote:

> Dear Respected Editor,
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> through this email we attach the results of the repairs that we have worked on with the team. thank you for your wisdom.
>
> Best Regards,
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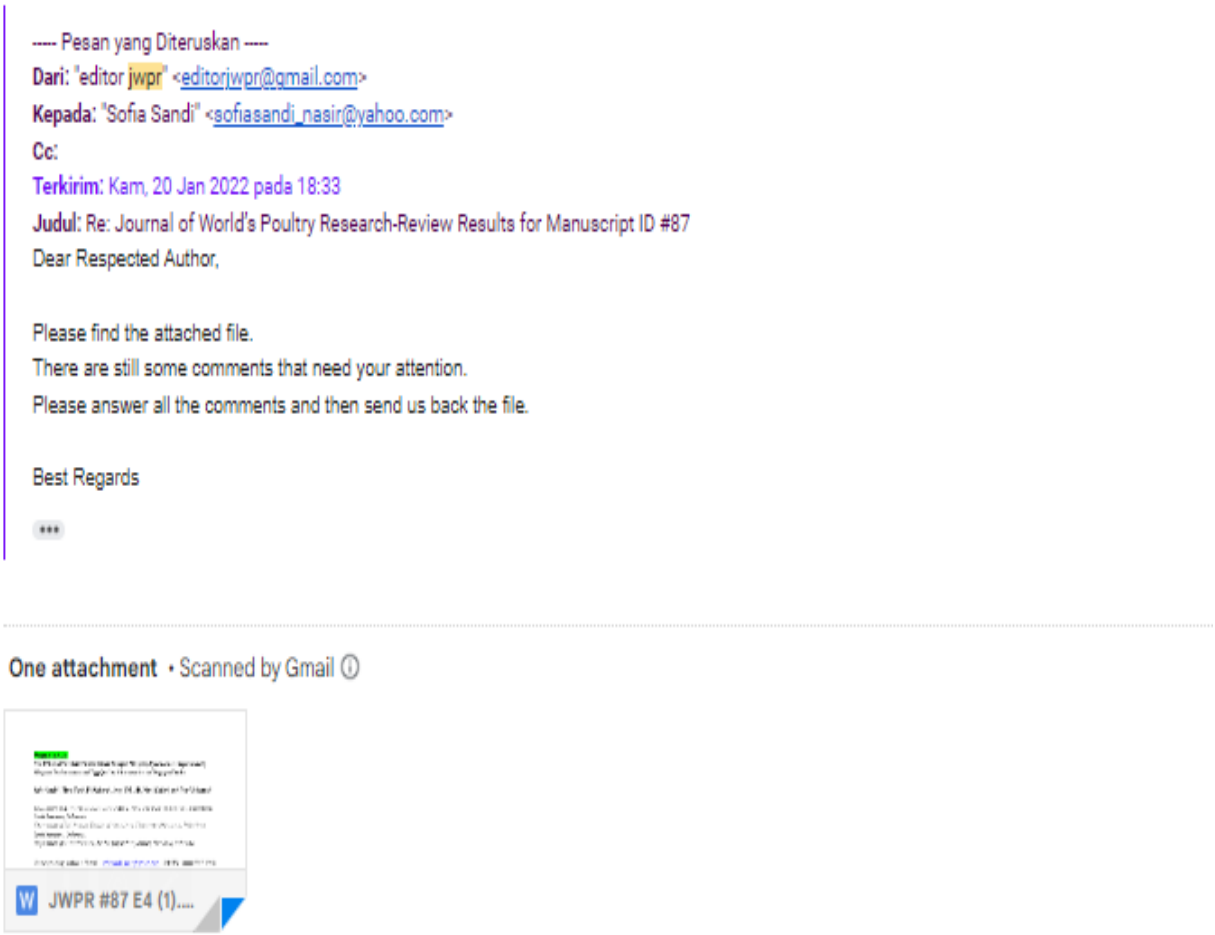
> Sofia Sandi

7. Bukti konfirmasi Hasil review ke-tiga (22 Januari 2022)



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Kepada: "Sofia Sandi" <sofiasandi_nasir@yahoo.com>
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Original Article

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

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Abstract

The study aimed to determine the effect of probiotic derived from an isolate of silage Kumpai Minyak grass 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 were diet without treatment (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) has established a significant effect on egg weight, compared to other treatments. The same results were also shown in daily egg production and feed conversion ratio, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment. Different results were found in the observations on feed consumption, where the overall treatment diet showed 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, other observations showed that egg index, egg yolk index, shell weight, and thickness are inversely related to the variables. The The probiotic treatment at the level of 0.8% was due to its ability to inhibit pathogenic bacteria and optimize the digestive tract, thus increasing digestibility and absorption of feed nutrients. 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.

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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 meaning that they can produce meat and eggs. Sari et al. (2011) reported that the average weight of Pegagan duck eggs was over 70 grams, and this value was relatively high compared to other local duck eggs. However, the large potential of Pegagan duck is not supported by a good maintenance system, which is still traditional/extensive so that the productivity is still very low due to the high risk of disease and insufficient nutrient needs (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 (Amine et al., 2020; Raphael et al., 2017). 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 (El-Kholy et al., 2020; Sandi et al., 2019).

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 probiotics 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. Similarly, Sandi et al. (2019) reported that Lactic acid bacteria (LAB) isolated from Kumpai grass silage as a probiotic and tested *in vitro* showed resistance implying 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 physical quality of the eggs. Based on this description, a study was conducted onthe 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 probiotics used in this study 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. As for the selected female ducks, they were already in the laying phase and had physical characteristics of a 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 m^2. 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 2021. 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

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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 with 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.

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 are 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. Apparatenfabreik 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):

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

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 that have been carried out 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 probiotic treatment 0.8% (P8) has established a considerable effect on egg weight compared to other treatments ($p < 0.05$). The same results were also shown in daily egg production and FCR, where there was a significant effect on 0.8% (P8) probiotic treatment compared to 0.2% (P2) and 0.4% (P4) probiotic treatment ($p < 0.05$). However, it was not significantly different compared with the control treatment and the probiotic 0.6% (P6) ($p <-> 0.05$). Different results were found in the observations on feed consumption, where the overall treatment diet showed significant results compared to the control treatment ($p < 0.05$) and did not differ significantly between probiotic treatments ($p > 0.05$).

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Analysis of performance data, including egg weight and daily egg production, is often tested on laying birds. 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 is used as a benchmark to determine absorbed nutrients and is used by 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.

~~In this study~~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-Khalaifa 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, Sjofjan 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.

Egg quality

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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. ~~Furthermore, the results were inversely proportional to the variable egg index, egg yolk index, shell weight, and thickness.~~

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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 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 consumption 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; Park et al., 2016; Bidura et al., 2019; Mikulski et al., 2020; Selim and Hussein, 2020).

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Conclusion

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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.

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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~~ 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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Table 1. Nutrient composition of diets of Pegagan Ducks

Ingredients	Amount (g/kg)
Maize (Corn)	484
Rice bran	185
Meat Bone Meal (MBM)	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

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^{e}	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: Standart 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: Standart error means
^{abc} Means in the same row without common letter are different at $p < 0.05$

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8. Bukti konfirmasi artikel accepted

Scienceline Pub.
www.science-line.com

Journal of World's Poultry Research
ISSN 2322-455X
www.jwpr.science-line.com

Dated: January 30, 2022

Paper Acceptance Certificate

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We are pleased to inform you that your manuscript **JWPR #87** entitled **"The Effect of Probiotic Derived from Kumpai Minyak (*Hymenachne Amplexicaulis*) Silage on Performance and Egg Quality Characteristcs of Pegagan Ducks"** has been reviewed by our selected reviewers/editors and accepted as **Original Research** for publication in the Journal of World's Poultry Research.

Thank you for submitting your manuscript to JWPR. Should you have any questions, please contact us.

Sincerely,
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Editor-in-Chief
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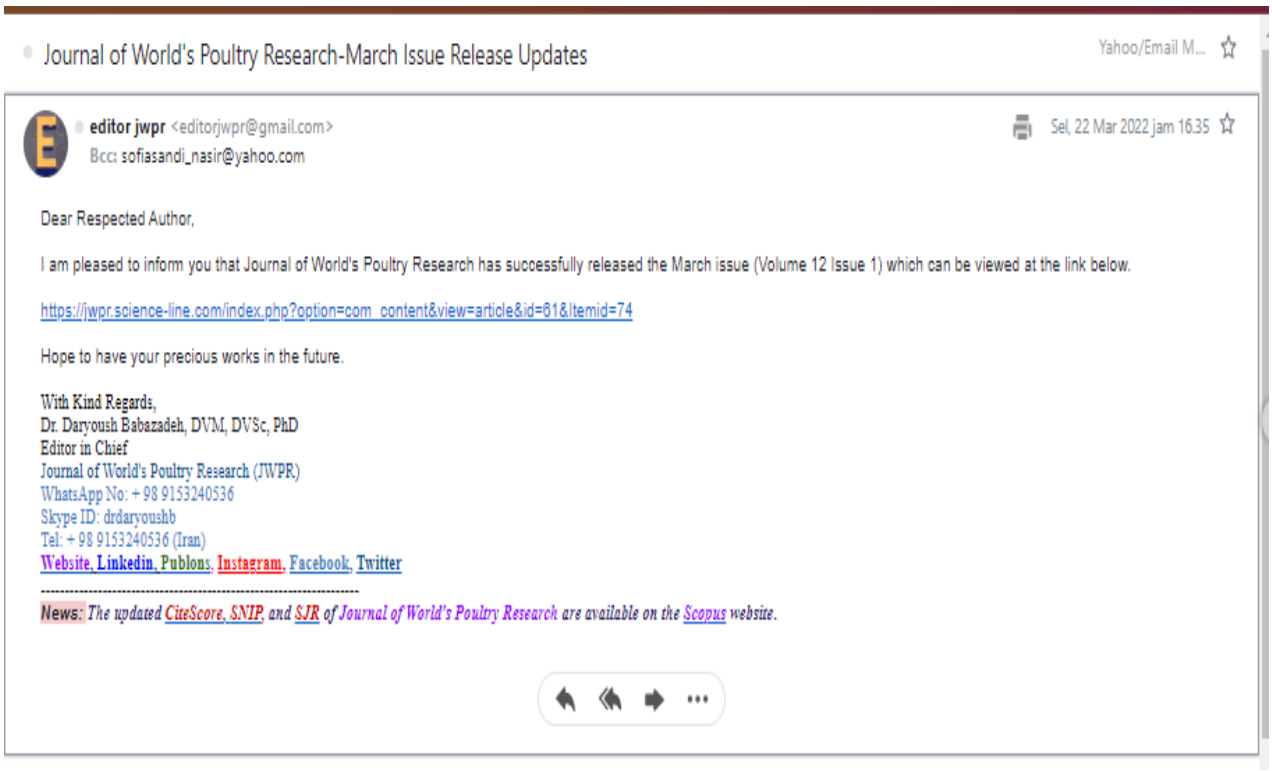
9. Bukti konfirmasi artikel published online (22 Maret 2022)



Journal of World's Poultry Research-March Issue Release Updates

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Sel, 22 Mar 2022 jam 16.35

Dear Respected Author,

I am pleased to inform you that Journal of World's Poultry Research has successfully released the March issue (Volume 12 Issue 1) which can be viewed at the link below.

https://jwpr.science-line.com/index.php?option=com_content&view=article&id=61&Itemid=74

Hope to have your precious works in the future.

With Kind Regards,
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