BUKTI KORESPONDENSI
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel : Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck

Jurnal : Tropical Animal Health and Production, 2022, 54(1), 65

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No	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit 1	27 April 2021
2	Bukti konfirmasi review dan hasil review pertama	28 April 2021
3	Bukti konfirmasi review dan hasil review kedua	30 Juni 2021
4	Bukti konfirmasi submit artikel dan artikel yang disubmit 3	14 Juli 2021
5	Bukti konfirmasi review dan hasil review	10 September 2021
6	Bukti submit artikel 4	2 Oktober 2021
7	Bukti konfirmasi review ke full artikel ke research note	4 Desember 2021
8	Bukti konfirmasi artikel accepted	4 Januari 2022
9	Bukti konfirmasi artikel published online	18 Januari 2022

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Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck

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Manuscript Number:	TROP-D-21-00659
Full Title:	Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck
Article Type:	Regular Articles
Keywords:	Probiotics; Organic Acid Salt; Egg Quality; Pegagan Duck
Corresponding Author:	asep indra munawar ali, Ph.D. Universitas Sriwijaya Fakultas Pertanian Ogan Ilir, None Selected INDONESIA
Corresponding Author Secondary Information:	
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Funding Information:	Universitas Sriwijaya (096/SP2H/LT/DRPM/IV/2019) — mrs. Sofia Sandi
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Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck

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Abstract

The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. It used 72 Pegagan laying ducks, 24 weeks old which randomly allocated to six treatment groups: basal diet, basal diet+organic acid, basal diet+probiotic, basal diet+tetracycline, basal diet+probiotic+organic acid, and basal diet+organic acid+tetracycline. The result showed that the feeding containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk color score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significant ($P > 0.05$) different. In conclusion, the best of egg quality in this study was in the dietary treatment of basal diet+probiotic+organic acid with the highest yolk color score and the lowest yolk fat and cholesterol content (11.92, 24.08%, and 284.75 mg/dl, respectively).

Keyword Probiotics, Organic Acid Salt, Egg Quality, Pegagan Duck

Introduction

Probiotic is live microbial feed supplements that positively influence the balance of microorganisms in the digestive tract of host animals (Fuller, 1989; Abdelqader et al., 2013). The dietary suplementation of probiotics has been found to improve growth performance and feed conversion ratio of broilers (Patel et al., 2015), egg size, egg mass, egg weight (Jin et al., 1997) and reduce egg yolk cholesterol in laying hen (Mohan et al., 1995;

Abdulrahim et al., 1996) and meat quality of broiler chickens (Pelicano et al., 2003). The utilization of probiotics could improve the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the animal host by stimulating the synthesis of vitamins B-group, improving host immunity, and increasing volatile fatty acids. The improvement in digestive tracts resulted in an increase in intake and nutrient digestibility (Fuller and Cadenhead, 1991; Jin et al., 1997; Rolfe, 2000).

Organic acids have been used for a long time as feed additives and commonly referred to as acidifiers or acidifying agents that have shown favorable effects on the nutrient utilization. Dietary supplementation with organic acids was associated with lowering the pH of the digestive tract and related to reductions of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke, 2003). The supplementation of organic acids also improved feed efficiency, egg mass, egg shell quality, and yolk index in layer hen (Soltan, 2008).

Lowland farming serves potential contributions for the sustainability of food production in South Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic flooding during the rainy season. The Pegagan duck is considered more suitable for small-scale farmers in the region. The waterfowl provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah, 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to the duck egg quality parameters have not been tested. Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the effectiveness of supplements and antibiotic tetracycline.

Materials and Methods

Study site

An animal feeding experiment was conducted for 10 weeks 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.

Experimental animal and treatments

Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate. The ducks were kept in experimental pens (1.5 m^2, four birds in each pen) with 5 cm of rice husk litter. The pens were located in an open-sided barn after proper cleaning and disinfection. The animals were fed with a basal diet (NRC, 1994) that was processed as crumble. The composition and nutrient concentrations of the basal diet are shown in Table 1. The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water were provided ad libitum while the lighting was provided 24 h.

Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2 %, v/v) was added in drinking water. The probiotic preparation was referred to the method of Bregni et al. (2000). The silage was prepared from a local swamp grass (Kumpai Tembaga grass, *Hymenachne acutigluma*) plus 3% molasses (w/w, fresh matter basis) (Sandi et al., 2018). An isolate from the silage was incubated in the deMann Rogosa Sharp medium for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the supernatant and substrate. An amount of 5% (w/w) of the mixture of milk skim and maltodextrin was added to the substrate. Afterward, the substrate was dried at 37 ^oC for 48 hours. Organic acid preparation was referred to Negara (2009): The distilled water was added to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered and stored in the refrigerator (5 ^oC) before use.

Sampling and handling

Feed offered was sampled each week and then pooled at the end of the experiment. Seven eggs from each replicate at the end of this study were randomly selected and later analyzed for egg quality (egg weight, albumen weight, yolk weight, eggshell weight, yolk color, egg index, albumen index, yolk index, and Haugh unit). The other three eggs from each replicate were randomly selected and later analyzed for the content of water, crude protein, crude fat, and yolk cholesterol in eggs.

Laboratory analyses and measurement methods

Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, extract ether, calcium, phosphor, and potassium according to the methods of AOAC (2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance (Ohaus CP214). Yolk color score, egg index, yolk index, albumen index, Haugh unit were measured according to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg quality parameters were measured in all cases within 48 hours of collection.

Statistical analysis

Data generated in the present study were subjected to statistical analysis using one-way ANOVA (R Core Team, 2018) in a completely randomized design. Normality of residuals data distribution was tested by Kolmogorov-Smirnov test. All data were reported as the arithmetic mean. Tukey test was used to separate significant treatment means, and significance was declared if $P < 0.05$.

Result and discussion

The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio (Data not shown). Eight weight, albumen weight, and yolk weight were also not different ($P > 0.05$) among the treatments while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic treatments compare to the basal diet treatment (Table 2). The range of the eggshell weight in the present study was similar to the range of study of Sumiati et al. (2020) in egg of local Pajajaran duck (7.97 to 8.46) but higher than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained in the organic acid + probiotic supplementation. Though the present study did not measure calcium and phosphor content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphor in the lower pH of the digestive tracts (Soltan, 2008; Abbas et al., 2013). Supplementation of the mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability of calcium and phosphor due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the minerals retention and bone mineralization (Ziaie et al., 2011).

The yolk color was affected ($P < 0.01$) by the treatments (Table 2). The yolk color score ranged from 10.41 to 11.92 which higher than that in the egg of the Pajajaran duck (5.56 to 8.89). The score in the present work could be categorized as good quality egg according to Sudaryani (2000) where the good color score range from 9.00 to 12.00. The highest mean of the color index was found in the organic acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might relate to a higher absorption of beta carotene. The yolk index of the duck in this study ranged from 0.38 to 0.41. The range was lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al., 2012). Yolk index is mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index value was in line with the protein contents of the egg in the present study where the highest protein content was also found in the organic acid+probiotic treatment while the lowest in the basal diet.

The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and cholesterol content in the yolk ($P < 0.01$). The range of Haugh unit score in the present work was similar to the range in the study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic acid+probiotic supplementation (Table 2). The decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study by Li et al. (2011) was also reported that supplementation of *Bacillus subtilis* in the diet of Shaoxing duck reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L. The role of probiotics in reducing egg cholesterol could be related to the changing lipid metabolism in the digestive tract. According to previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid.

Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat, and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the highest yolk color index was found in organic acid+probiotic supplementation. The yolk fat and cholesterol content were lower than those in the other supplementations.

Acknowledgements The authors are grateful to Universitas Sriwijaya for the financial support of this research.

Author contribution Original intellectual concept and study design: Sofia Sandi; Methodology: Fitra Yosi, Meisji L. Sari; Data curation, formal analysis, and investigation: Eli Sahara, Bella P. Maharani, Asmak; Writing - original draft preparation: Asep I. M. Ali; Writing - review and editing: Muhamad N. Rofiq, Asep I. M. Ali; Funding acquisition: Sofia Sandi. All authors read and approved the final manuscript.

Funding The research received funding from a competitive grant (number 096/SP2H/LT/DRPM/IV/2019).

Data availability on request.

Code availability not applicable

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Consent to participate not applicable

Consent for publication not applicable

Conflict of interest the authors declare that they have no competing interest.

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Table 1 Composition and nutrients content of the basal feed (dry matter basis)

Ingredients	**%**	**Nutrient**	**%**
Maize grain	43.00	Metabolizable energy	2771.08*
Rice bran	19.80	Crude protein	23.97
Comercial concentrate	19.10	Crude fiber	9.64
Soybean meal	12.20	Ether extract	5.91
Meat bone meal	4.90	Calcium	2.86
Methionine	0.40	Phosphor	1.08
Lysine	0.60	Potassium	0.45

*Kcal/kg, calculated based on NRC (1994)

Table 2 Effect of feeding containing probiotic, organic acid, and tetracycline on egg quality of Pegagan ducks

Parameters	Experimental treatments						SEM
	Basal	Basal + organic acid	Basal + probiotik	Basal + tetracycline	Basal + organic acid + probiotic	Basal + organic acid + tetracycline	
Egg weight (g)	59.10	60.12	62.56	61.81	63.24	61.40	0.562
Albumen weight (g)	31.15	31.78	33.9	32.71	34.85	32.44	0.526
Yolk weight (g)	17.41	17.68	18.79	17.89	19.11	18.48	0.355
Eggshell weight (g)	7.64[b]	7.87[ab]	8.83[a]	8.53[ab]	8.96[a]	7.86[ab]	0.166
Yolk color score	10.41[b]	10.75[b]	10.83[b]	10.79[b]	11.92[a]	10.83[b]	0.130
Egg index	71.23[c]	72.97[bc]	76.07[b]	74.65[bc]	79.36[a]	73.19[bc]	0.753
Albumen index	0.12	0.13	0.13	0.13	0.14	0.13	0.003
Yolk index	0.38	0.39	0.41	0.41	0.42	0.40	0.008
Haugh unit (HU)	88.80[c]	90.10[b]	92.34[ab]	91.27[ab]	92.44[a]	91.10[ab]	0.369
Water Content (%)	63.07	62.48	61.97	62.79	59.33	61.55	0.495
Protein (%)	12.25	13.11	13.56	12.73	14.02	12.99	0.201
Yolk Fat (%)	27.09[a]	26.20[ab]	26.09[b]	26.21[ab]	24.09[c]	24.99[bc]	0.253
Yolk Cholesterol (mg/dl)	465.26[a]	327.76[b]	313.99[b]	348.01[b]	284.75[c]	306.62[b]	14.260

SEM: Standard error of mean

Within rows, means with different superscripts differ at $p < .05$ (Tukey post hoc test).

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Tropical Animal Health and Production

Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck

--Manuscript Draft--

Manuscript Number:	TROP-D-21-00659R1
Full Title:	Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck
Article Type:	Regular Articles
Corresponding Author:	asep indra munawar ali, Ph.D. Universitas Sriwijaya Fakultas Pertanian Ogan Ilir, None Selected INDONESIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universitas Sriwijaya Fakultas Pertanian
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Funding Information:	Universitas Sriwijaya (096/SP2H/LT/DRPM/IV/2019) — mrs. Sofia Sandi

Reviewer Comments		Authors Responses	
Comments	**Page & line**	**Comments/Correction**	**New Page & line**
The authors haven't mentioned anything about why they wanted to use the probiotics derived from grass silage, neither there is any mention about which bacteria is present in the current probiotic culture isolated from the grass silage. The authors need to mention the importance of using probiotics derived from grass silage		The reasons for using of probiotic derived from grass silage have been added	2 & 54
When water is already acidic, why authors have supplemented acidifiers/organic acids and that too which organic acid has not been mentioned. It again needs to be mentioned and use of acidifiers needs to be justified.		The sentence has been added	2 & 54
Why 4 birds in one pen? When each replicate contains 3 birds, why not separate pen for each replicate? If authors have written this by mistake, they need to correct it.	3 & 71	The number of birds has been corrected	3 & 70
Is the light required for fully grown birds during day hours?	3 & 76	The sentence has been corrected	3 & 75
Composition of organic acid and probiotic needs to be mentioned.		The compositions have been added	3 & 81 3 & 85
Calcium & Phosphorus measurement methods need to be separate		The methods has been separated	4 & 96
Please mention "7 fresh eggs"		It has been mentioned	4 & 89
The discussion part related to fat and cholesterol content of eggs needs to be strengthened more. The current discussion and justifications seem to be insufficient to reach the conclusions given.		The discussion has been strengthened	5 &132-135 5 & 141-147 6 & 148
Add one column and mention P value	Table 2	The P values have been added	Table 2
		Thank you so much for your correction and suggestions	

Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck

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Abstract

The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. 72 Pegagan laying ducks (Average age: 24 weeks) were randomly allocated to six treatment groups: basal diet, basal diet+organic acid, basal diet+probiotic, basal diet+tetracycline, basal diet+probiotic+organic acid, and basal diet+organic acid+tetracycline. The result showed that the feeding containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk colour score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significantly ($P > 0.05$) different. In conclusion, the best of egg quality in this study was in the dietary treatment of basal diet+probiotic+organic acid with the highest yolk colour score and the lowest yolk fat and cholesterol content.

Keyword Probiotics, Organic Acid Salt, Egg Quality, Pegagan Duck

Introduction

Probiotic is live microbial feed supplements that positively influences the balance of microorganisms in the digestive tract of host animals (Fuller, 1989; Abdelqader et al., 2013). The dietary suplementation of probiotics has been found to improve growth performance and feed conversion ratio of broilers (Patel et al., 2015), egg size, egg mass, egg weight (Jin et al., 1997); reduce egg yolk cholesterol in laying hen (Mohan et al., 1995; Abdulrahim et al., 1996) and meat quality of broiler chickens (Pelicano et al., 2003). The utilization of probiotics could improve the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal by stimulating the synthesis of vitamins B-group, improving immunity, and increasing volatile fatty acids. The improvement in digestive tracts resulted in an increase in intake and nutrient digestibility (Fuller and Cadenhead, 1991; Jin et al., 1997; Rolfe, 2000).

Organic acids have been used for a long time as feed additives that have shown favorable effects on the nutrient utilization. An increase of pancreatic secretion has been reported (Dibner and Buttin, 2002) while Adil et al. (2010) reported that the addition of organic increased villus height in the small intestines and serum calcium and phosphorus concentrations. Dietary supplementation with organic acids was associated with lowering the pH of the digestive tract and related to reduction of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke, 2003). The supplementation of organic acids also improved feed efficiency, egg mass, egg shell quality, and yolk index in layer hen (Soltan, 2008).

Lowland farming serves potential contributions for the sustainability of food production in South Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic flooding during the rainy season. The Pegagan duck is considered more suitable for small-scale farmers in the region. The waterfowl provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah, 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to the duck egg quality parameters have not been tested. Organic acids and lactic acid bacteria derived from grass silage might still serve as a valuable alternative of additive since simple and less expensive preparation (Sandi et al., 2020). The organic acids could serve not only for lowering pH when the digestive tracts were already acidic. Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the effectiveness of supplements and antibiotic tetracycline.

Materials and Methods

Study site

An animal feeding experiment was conducted for 10 weeks 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.

Experimental animal and treatments

Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate. The ducks were kept in experimental pens (1.5 m^2, three birds in each pen) with 5 cm of rice husk litter. The pens were located in an open-sided barn after proper cleaning and disinfection. The birds were fed with a basal diet (NRC, 1994) that was processed as crumble. The composition and nutrient concentrations of the basal diet are shown in Table 1. The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.

Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2 %, v/v) was added in drinking water. The probiotic preparation was referred to the method of Bregni et al. (2000). The silage was prepared from a local swamp grass (Kumpai Tembaga grass, *Hymenachne acutigluma*) plus 3% molasses (w/w, fresh matter basis) (Sandi et al., 2018). An isolate from the silage was incubated in the deMann Rogosa Sharp medium for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the supernatant and substrate. The substrate contained 8.24 colony-forming unit of lactic acid bacteria per ml. An amount of 5% (w/w) of the mixture of milk skim and maltodextrin was added to the substrate. Afterward, the substrate was dried at 37 ^{o}C for 48 hours. Organic acid preparation was referred to Negara (2009): The distilled water was added to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered and stored in the refrigerator (5 ^{o}C) before use. Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of acetic acid (v/v).

Sampling and handling

Feed offered was sampled each week and then pooled at the end of the experiment. Seven fresh eggs from each replicate at the end of this study were randomly selected and later analyzed for egg quality (egg weight, albumen weight, yolk weight, eggshell weight, yolk color, egg index, albumen index, yolk index, and Haugh unit). The other three eggs from each replicate were randomly selected and later analyzed for the content of water, crude protein, crude fat, and yolk cholesterol in eggs.

Laboratory analyses and measurement methods

Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract ether (AOAC, 2005). Calcium and potassium oncentrations were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800 while phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer Lambda 45 (AOAC, 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were measured according to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg quality parameters were measured in all cases within 48 hours of collection.

Statistical analysis

Data generated in the present study were subjected to statistical analysis using one-way ANOVA (R Core Team, 2018) in a completely randomized design. Normality of residuals data distribution was tested by Kolmogorov-Smirnov test. All data were reported as the arithmetic mean. Tukey test was used to separate significant treatment means, and significance was declared if $P < 0.05$.

Result and discussion

The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio (Data not shown). Eight weight, albumen weight, and yolk weight were also not different ($P > 0.05$) among the treatments while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic treatments compared to the basal diet treatment (Table 2). The range of the eggshell weight in the present study was similar to the range of study of Sumiati et al. (2020) in egg of local Pajajaran duck (7.97 to 8.46 g) but higher than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained in the organic acid + probiotic supplementation. Though the present study did not measure calcium and phosphorus content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphorus in the lower pH of the digestive tracts (Soltan, 2008; Abbas et al., 2013). Supplementation of the mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability of calcium and phosphorus due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the minerals retention and bone mineralization (Ziaie et al., 2011).

The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score ranged from 10.41 to 11.92 which was higher than that in the egg of the Pajajaran duck (5.56 to 8.89). The score in the present work could be categorized as good quality egg according to Sudaryani (2000) where the good color score range from 9.00 to 12.00. The highest mean of the colour index was found in the organic acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might related to a higher absorption of beta carotene. The yolk index of the duck in this study ranged from 0.38 to 0.41. The range was lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al., 2012). Yolk index is mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index value was in line with the protein contents of the egg in the present study where the highest protein content was also found in the organic acid+probiotic treatment while the lowest in the basal diet.

The organic acid supplementation improved Haugh unit and the cholesterol content in the yolk ($P < 0.05$) though the drinking water was already acidic. The lactic and acetic acid in the acid supplementation could improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the previous studies reported ((Mohan et al., 1995; Abdulrahim et al., 1996).

The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and cholesterol content ($P < 0.01$). The range of Haugh unit score in the present work was similar to the range in the study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic acid+probiotic supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a result of biological and chemical activity produced by the organic acid+probiotic in the digestive tract. According to previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liong, (2010) reviewed that three mechanisms could cause the decrease in cholesterol and fat levels: 1) inhibition of cholesterol synthesis due to probiotic fermented compounds, 2) bile salt deconjugation, which causes a higher use of cholesterol for bile salt synthesis, and 3) the ability of probiotics to bind cholesterol. Furthermore, the decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study by Li et al. (2011) also reported that supplementation of *Bacillus subtilis* in the diet of Shaoxing duck reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L.

Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat, and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the highest yolk color index was found in organic acid+probiotic supplementation. The yolk fat and cholesterol content were lower than those in the other supplementations.

Acknowledgements The authors are grateful to Universitas Sriwijaya for the financial support of this research. We sincerely thank an anonymous reviewer for critically reading and suggesting substantial improvements.

Author contribution Original intellectual concept and study design: Sofia Sandi; Methodology: Fitra Yosi, Meisji L. Sari; Data curation, formal analysis, and investigation: Eli Sahara, Bella P. Maharani, Asmak; Writing - original draft preparation: Asep I. M. Ali; Writing - review and editing: Muhamad N. Rofiq, Asep I. M. Ali; Funding acquisition: Sofia Sandi. All authors read and approved the final manuscript.

Funding The research received funding from a competitive grant (number 096/SP2H/LT/DRPM/IV/2019).

Data availability on request.

Code availability not applicable

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Consent to participate not applicable

Consent for publication not applicable

Conflict of interest the authors declare that they have no competing interest.

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Table 1 Composition and nutrients content of the basal feed (dry matter basis)

Ingredients	**%**	**Nutrient**	**%**
Maize grain	43.00	Metabolizable energy	2771.08*
Rice bran	19.80	Crude protein	23.97
Comercial concentrate	19.10	Crude fiber	9.64
Soybean meal	12.20	Ether extract	5.91
Meat bone meal	4.90	Calcium	2.86
Methionine	0.40	Phosphorus	1.08
Lysine	0.60	Potassium	0.45

*Kcal/kg, calculated based on NRC (1994)

Table 2 Effect of feeding containing probiotic, organic acid, and tetracycline on egg quality of Pegagan ducks

Parameters	Experimental treatments						SEM	*P* value
	Basal	Basal + organic acid	Basal + probiotic	Basal + tetracycline	Basal + organic acid + probiotic	Basal + organic acid + tetracycline		
Egg weight (g)	59.10	60.12	62.56	61.81	63.24	61.40	0.562	0.291
Albumen weight (g)	31.15	31.78	33.9	32.71	34.85	32.44	0.526	0.365
Yolk weight (g)	17.41	17.68	18.79	17.89	19.11	18.48	0.355	0.749
Eggshell weight (g)	7.64[b]	7.87[ab]	8.83[a]	8.53[ab]	8.96[a]	7.86[ab]	0.166	0.035
Yolk color score	10.41[b]	10.75[b]	10.83[b]	10.79[b]	11.92[a]	10.83[b]	0.130	0.008
Egg index	71.23[c]	72.97[bc]	76.07[b]	74.65[bc]	79.36[a]	73.19[bc]	0.753	0.014
Albumen index	0.12	0.13	0.13	0.13	0.14	0.13	0.003	0.959
Yolk index	0.38	0.39	0.41	0.41	0.42	0.40	0.008	0.873
Haugh unit (HU)	88.80[c]	90.10[b]	92.34[ab]	91.27[ab]	92.44[a]	91.10[ab]	0.369	0.017
Water Content (%)	63.07	62.48	61.97	62.79	59.33	61.55	0.495	0.288
Protein (%)	12.25	13.11	13.56	12.73	14.02	12.99	0.201	0.149
Yolk Fat (%)	27.09[a]	26.20[ab]	26.09[b]	26.21[ab]	24.09[c]	24.99[bc]	0.253	0.001
Yolk Cholesterol (mg/dl)	465.26[a]	327.76[b]	313.99[b]	348.01[b]	284.75[c]	306.62[b]	14.260	0.000

SEM: Standard error of mean

Within rows, means with different superscripts differ at $P < .05$ (Tukey post hoc test).

Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck

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Abstract

The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. ~~It used~~ 72 Pegagan laying ducks~~,~~ (Average age: 24 weeks~~)~~ ~~old which~~ were randomly allocated to six treatment groups: basal diet, basal diet+organic acid, basal diet+probiotic, basal diet+tetracycline, basal diet+probiotic+organic acid, and basal diet+organic acid+tetracycline. The result showed that the feeding containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk colour score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significantly ($P > 0.05$) different. In conclusion, the best of egg quality in this study was in the dietary treatment of basal diet+probiotic+organic acid with the highest yolk colour score and the lowest yolk fat and cholesterol content ~~(11.92, 24.08%, and 284.75 mg/dl, respectively)~~.

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Keyword Probiotics, Organic Acid Salt, Egg Quality, Pegagan Duck

Introduction

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Probiotic is live microbial feed supplements that positively influences the balance of microorganisms in the digestive tract of host animals (Fuller, 1989; Abdelqader et al., 2013). The dietary suplementation of probiotics has been found to improve growth performance and feed conversion ratio of broilers (Patel et al., 2015), egg size, egg mass, egg weight (Jin et al., 1997); ~~and~~ reduce egg yolk cholesterol in laying hen (Mohan et al., 1995;

Abdulrahim et al., 1996) and meat quality of broiler chickens (Pelicano et al., 2003). The utilization of probiotics could improve the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal ~~host~~ by stimulating the synthesis of vitamins B-group, improving ~~host~~ immunity, and increasing volatile fatty acids. The improvement in digestive tracts resulted in an increase in intake and nutrient digestibility (Fuller and Cadenhead, 1991; Jin et al., 1997; Rolfe, 2000).

Organic acids have been used for a long time as feed additives ~~and commonly referred to as acidifiers or acidifying agents~~ that have shown favorable effects on the nutrient utilization. An increase of pancreatic secretion has been reported (Dibner and Buttin, 2002) while Adil et al. (2010) reported that the addition of organic increased villus height in the small intestines and serum calcium and phosphorus concentrations. Dietary supplementation with organic acids was associated with lowering the pH of the digestive tract and related to reduction~~s~~ of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke, 2003). The supplementation of organic acids also improved feed efficiency, egg mass, egg shell quality, and yolk index in layer hen (Soltan, 2008).

Lowland farming serves potential contributions for the sustainability of food production in South Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic flooding during the rainy season. The Pegagan duck is considered more suitable for small-scale farmers in the region. The waterfowl provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah, 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to the duck egg quality parameters have not been tested. ~~When the digestive tract of the duck was presumably acidic, Oo~~Organic acids and lactic acid bacteria derived from ~~a~~ grass silage might still serve as a valuable alternative of additive ~~for an improvement of the digestive health and absorption~~since simple and less expensive preparation (Sandi et al., 2020). The organic acids could serve not only for lowering pH when the digestive tracts were already acidic.- Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and

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organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the effectiveness of supplements and antibiotic tetracycline.

Materials and Methods

Study site

An animal feeding experiment was conducted for 10 weeks 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.

Experimental animal and treatments

Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate. The ducks were kept in experimental pens (1.5 m^2, ~~four~~ three birds in each pen) with 5 cm of rice husk litter. The pens were located in an open-sided barn after proper cleaning and disinfection. The ~~animals~~ birds were fed with a basal diet (NRC, 1994) that was processed as crumble. The composition and nutrient concentrations of the basal diet are shown in Table 1. The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.~~24 h.~~

Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2 %, v/v) was added in drinking water. The probiotic preparation was referred to the method of Bregni et al. (2000). The silage was prepared from a local swamp grass (Kumpai Tembaga grass, *Hymenachne acutigluma*) plus 3% molasses (w/w, fresh matter basis) (Sandi et al., 2018). An isolate from the silage was incubated in the deMann Rogosa Sharp medium for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the supernatant and substrate. The substrate contained ~~had~~ 8.24 colony-forming unit/~~ml~~ of lactic acid bacteria per ml. An amount of 5% (w/w) of the mixture of milk skim and maltodextrin was added to the substrate. Afterward, the substrate was dried at 37 ºC for 48 hours. Organic acid preparation was referred to Negara (2009): The distilled water was added to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered and stored in the refrigerator (5 ºC) before use. Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of acetic acid (v/v).

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Sampling and handling

Feed offered was sampled each week and then pooled at the end of the experiment. Seven fresh eggs from each replicate at the end of this study were randomly selected and later analyzed for egg quality (egg weight, albumen weight, yolk weight, eggshell weight, yolk color, egg index, albumen index, yolk index, and Haugh unit). The other three eggs from each replicate were randomly selected and later analyzed for the content of water, crude protein, crude fat, and yolk cholesterol in eggs.

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Laboratory analyses and measurement methods

Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract ether (AOAC, 2005)~~, calcium, phosphorus, and potassium according to the methods of AOAC (2005)~~. Calcium and potassium concentrations ~~of Ca and Mg~~ were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800 while ~~P~~phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer Lambda 45 (AOAC~~e~~, 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were measured according to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg quality parameters were measured in all cases within 48 hours of collection.

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Statistical analysis

Data generated in the present study were subjected to statistical analysis using one-way ANOVA (R Core Team, 2018) in a completely randomized design. Normality of residuals data distribution was tested by Kolmogorov-Smirnov test. All data were reported as the arithmetic mean. Tukey test was used to separate significant treatment means, and significance was declared if $P < 0.05$.

Result and discussion

The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio (Data not shown). Eight weight, albumen weight, and yolk weight were also not different ($P > 0.05$) among the treatments while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic treatments compared to the basal diet treatment (Table 2). The range of the eggshell weight in the present study was similar to the range of study of Sumiati et al. (2020) in egg of local Pajajaran duck (7.97 to 8.46 g) but higher than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained in the organic acid + probiotic supplementation. Though the present study did not measure calcium and

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phosphorus content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphorus in the lower pH of the digestive tracts (Soltan, 2008; Abbas et al., 2013). Supplementation of the mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability of calcium and phosphorus due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the minerals retention and bone mineralization (Ziaie et al., 2011).

The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score ranged from 10.41 to 11.92 which was higher than that in the egg of the Pajajaran duck (5.56 to 8.89). The score in the present work could be categorized as good quality egg according to Sudaryani (2000) where the good color score range from 9.00 to 12.00. The highest mean of the colour index was found in the organic acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might related to a higher absorption of beta carotene. The yolk index of the duck in this study ranged from 0.38 to 0.41. The range was lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al., 2012). Yolk index is mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index value was in line with the protein contents of the egg in the present study where the highest protein content was also found in the organic acid+probiotic treatment while the lowest in the basal diet.

The organic acid supplementation improved Haugh unit and the cholesterol content in the yolk ($P < 0.05$) though the ~~. It seems that the acid~~ drinking water ~~might have less effect to the pH of the digestive tract but this needs further investigation~~ was already acidic. The lactic and acetic acid in the acid supplementation could improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the previous studies reported ((Mohan et al., 1995; Abdulrahim et al., 1996).

The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and cholesterol content ~~in the yolk~~ (P < 0.01). The range of Haugh unit score in the present work was similar to the range in the study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic acid+probiotic supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a result of ~~the~~ biological and chemical activity produced by the organic acid+probiotic ~~supplementation, where the accumulation of these two substrates causes changes in the lipid profile~~ in the digestive ~~system~~tract. According to previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liong, (2010) reviewed that ~~A study conducted to determine the effect of using probiotics on lowering cholesterol levels reported that~~ ~~tThere are 3 tT~~three mechanisms ~~that~~ couldan cause athe decrease in cholesterol and fat levels: 1)~~, namely 1).~~ inhibition of cholesterol synthesis due to probiotic fermented compounds,~~;~~ 2) ~~. B~~bile salt deconjugation, which causes a higher use of cholesterol for bile salt synthesis, and ~~and results in lower cholesterol levels;~~ 3) t~~. The existence of the~~ ability of probiotics to bind cholesterol~~, causing low cholesterol levels~~ . Furthermore, t~~T~~he decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study by Li et al. (2011) ~~was~~ also reported that supplementation of *Bacillus subtilis* in the diet of Shaoxing duck reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L. ~~The role of probiotics in reducing egg cholesterol could be related to the changing lipid metabolism in the digestive tract.~~ ~~According to previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid.~~

Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat, and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the highest yolk color index was found in organic acid+probiotic supplementation. The yolk fat and cholesterol content were lower than those in the other supplementations.

Acknowledgements The authors are grateful to Universitas Sriwijaya for the financial support of this research. We sincerely thank an anonymous reviewer for critically reading and suggesting substantial improvements.

Author contribution Original intellectual concept and study design: Sofia Sandi; Methodology: Fitra Yosi, Meisji L. Sari; Data curation, formal analysis, and investigation: Eli Sahara, Bella P. Maharani, Asmak; Writing - original draft preparation: Asep I. M. Ali; Writing - review and editing: Muhamad N. Rofiq, Asep I. M. Ali; Funding acquisition: Sofia Sandi. All authors read and approved the final manuscript.

Funding The research received funding from a competitive grant (number 096/SP2H/LT/DRPM/IV/2019).

Data availability on request.

Code availability not applicable

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Consent to participate not applicable

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Consent for publication not applicable

Conflict of interest the authors declare that they have no competing interest.

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Table 1 Composition and nutrients content of the basal feed (dry matter basis)

Ingredients	**%**	**Nutrient**	**%**
Maize grain	43.00	Metabolizable energy	2771.08*
Rice bran	19.80	Crude protein	23.97
Comercial concentrate	19.10	Crude fiber	9.64
Soybean meal	12.20	Ether extract	5.91
Meat bone meal	4.90	Calcium	2.86
Methionine	0.40	Phosphorus	1.08
Lysine	0.60	Potassium	0.45

*Kcal/kg, calculated based on NRC (1994)

Table 2 Effect of feeding containing probiotic, organic acid, and tetracycline on egg quality of Pegagan ducks

Parameters	Experimental treatments						SEM	*P* value
	Basal	Basal + organic acid	Basal + probiotic	Basal + tetracycline	Basal + organic acid + probiotic	Basal + organic acid + tetracycline		
Egg weight (g)	59.10	60.12	62.56	61.81	63.24	61.40	0.562	0.291
Albumen weight (g)	31.15	31.78	33.9	32.71	34.85	32.44	0.526	0.365
Yolk weight (g)	17.41	17.68	18.79	17.89	19.11	18.48	0.355	0.749
Eggshell weight (g)	7.64^{b}	7.87^{ab}	8.83^{a}	8.53^{ab}	8.96^{a}	7.86^{ab}	0.166	0.035
Yolk color score	10.41^{b}	10.75^{b}	10.83^{b}	10.79^{b}	11.92^{a}	10.83^{b}	0.130	0.008
Egg index	71.23^{c}	72.97^{bc}	76.07^{b}	74.65^{bc}	79.36^{a}	73.19^{bc}	0.753	0.014
Albumen index	0.12	0.13	0.13	0.13	0.14	0.13	0.003	0.959
Yolk index	0.38	0.39	0.41	0.41	0.42	0.40	0.008	0.873
Haugh unit (HU)	88.80^{c}	90.10^{b}	92.34^{ab}	91.27^{ab}	92.44^{a}	91.10^{ab}	0.369	0.017
Water Content (%)	63.07	62.48	61.97	62.79	59.33	61.55	0.495	0.288
Protein (%)	12.25	13.11	13.56	12.73	14.02	12.99	0.201	0.149
Yolk Fat (%)	27.09^{a}	26.20^{ab}	26.09^{b}	26.21^{ab}	24.09^{c}	24.99^{bc}	0.253	0.001
Yolk Cholesterol (mg/dl)	465.26^{a}	327.76^{b}	313.99^{b}	348.01^{b}	284.75^{c}	306.62^{b}	14.260	0.000

SEM: Standard error of mean

Within rows, means with different superscripts differ at $P < .05$ (Tukey post hoc test).

Asep Indra Munawar Ali fp <asep_ali@fp.unsri.ac.id>

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Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck: a research note

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Manuscript Number:	TROP-D-21-00659R3
Full Title:	Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck: a research note
Article Type:	Short Communications
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Funding Information:	Universitas Sriwijaya (096/SP2H/LT/DRPM/IV/2019) — mrs. Sofia Sandi

Reviewer Comments		Authors Responses	
Comments	**Page & line**	**Comments/Correction**	**New Page & line**
The authors haven't mentioned anything about why they wanted to use the probiotics derived from grass silage, neither there is any mention about which bacteria is present in the current probiotic culture isolated from the grass silage. The authors need to mention the importance of using probiotics derived from grass silage		The reasons for using of probiotic derived from grass silage have been added	2 & 50
When water is already acidic, why authors have supplemented acidifiers/organic acids and that too which organic acid has not been mentioned. It again needs to be mentioned and use of acidifiers needs to be justified.		The sentence has been added	2 & 51
Why 4 birds in one pen? When each replicate contains 3 birds, why not separate pen for each replicate? If authors have written this by mistake, they need to correct it.	3 & 71	The number of birds has been corrected	2 & 60
Is the light required for fully grown birds during day hours?	3 & 76	The sentence has been corrected	3 & 65
Composition of organic acid and probiotic needs to be mentioned.		The compositions have been added	3 & 75 3 & 71
Calcium & Phosphorus measurement methods need to be separate		The methods has been separated	3 & 82-84
Please mention "7 fresh eggs"		It has been mentioned	3 & 78
The discussion part related to fat and cholesterol content of eggs needs to be strengthened more. The current discussion and justifications seem to be insufficient to reach the conclusions given.		The discussion has been strengthened	4 & 117-120 5 & 126-137
Add one column and mention P value	Table 2	The P values have been added	Table 2
Kindly go through the manuscript and modify it in the light of comments inserted. And, this manuscript may considered as short communication rather than regular full length research article; as the data presented is limited.		The number of words has been reduced (3618 to 3118), about 4 pages of the journal	
Conclusion may be modified as below: It is concluded that dietary supplementation of organic acid and probiotic derived from grass silage improved egg quality in terms of yolk colour score, fat and cholesterol content.	Abstract 21-22		Abstract 21-22
Is this selective medium for growing lactic acid bacteria? If so, please mention in parenthesis. Also please mention the species of lactic acid bacteria that was present/expected to be present in the subtrate with 8.24 CFU.	82	Yes, selective medium. The spesies of lactic acid bacteria (*Lactobacillus plantarum*) has been inserted.	3 & 69 3 & 72
		All corrections on the body of the text. have been followed	
		Thank you so much for your correction and suggestions	
I would suggest changing article type from full length to research note and accept for publication		The number of word has been reduced and the title (as a research note) was mentioned	

Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck: a research note

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Abstract

The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. 72 Pegagan laying ducks (Average age: 24 weeks) were randomly allocated to six treatment groups: basal diet, basal diet+organic acid, basal diet+probiotic, basal diet+tetracycline, basal diet+probiotic+organic acid, and basal diet+organic acid+tetracycline. The result showed that the feeding diets containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk colour score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significantly ($P > 0.05$) different. It is concluded that dietary supplementation of organic acid and probiotic derived from grass silage improved egg quality in terms of yolk colour score, fat and cholesterol content.

Keyword Probiotics, Organic Acid Salt, Egg Quality, Pegagan Duck

Introduction

Probiotic suplementation has been found to improve growth performance and feed conversion ratio of broilers (Patel et al., 2015), egg size, egg mass, egg weight (Jin et al., 1997) and reduce egg yolk cholesterol in laying hen (Mohan et al., 1995; Abdulrahim et al., 1996). The utilization of probiotics could improve the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal by stimulating the synthesis of vitamins B-group, improving immunity, and increasing volatile fatty acids.

Pancreatic secretion was increased by organic acids supplementation (Dibner and Buttin, 2002). Villus height in the small intestines and serum calcium and phosphorus concentrations were also elevated (Adil et al., 2010). Organic acids supplementation was associated with lowering the pH of the digestive tract and related to reduction of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke, 2003). Moreover, organic acids supplementation improved feed efficiency, egg mass, eggshell quality, and yolk index in layer hen (Soltan, 2008).

Lowland serves potential contributions for the farming sustainability in South Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic flooding. The Pegagan duck is considered more suitable for small-scale farmers that provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah, 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to the duck egg quality parameters have not been tested. Organic acids and lactic acid bacteria derived from grass silage might serve as a valuable alternative of additive since simple and less expensive preparation (Sandi et al., 2020). The organic acids could serve not only for lowering pH when the digestive tracts were already acidic. Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the effectiveness of supplements and antibiotic tetracycline.

Materials and Methods

Experimental animal and treatments

An experimental trial was conducted for 10 weeks at the experimental station, Faculty of Agriculture, Universitas Sriwijaya. Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate. The ducks were kept in experimental pens (1.5 m^2, three birds in each pen) in an open-sided barn with 5 cm of rice husk litter after proper cleaning and disinfection. The birds were fed with a basal diet (NRC, 1994) that was processed as crumble (Table 1). The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.

Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2 %, v/v) was added in drinking water. The silage was prepared from a swamp grass (*Hymenachne acutigluma*) plus 3% molasses (w/w, fresh matter basis). An isolate from the silage was incubated in the deMann Rogosa Sharp medium (a selective medium for lactic acid bacteria) for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the supernatant and substrate. The substrate contained 8.24 colony-forming unit of *Lactobacillus plantarum* per ml. The substrate was mixed with an amount of 5% (w/w) of the mixture of milk skim and maltodextrin and then dried at 37 ºC for 48 hours (Sandi et al., 2018). Organic acid was prepared by mixing the distilled water to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered and stored in the refrigerator (5 ºC). Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of acetic acid (v/v) (Sandi et al., 2020).

Laboratory analyses and measurement methods

Feed offered was sampled each week and then pooled at the end of the experiment. Seven fresh eggs from each replicate at the end of this study were randomly selected and later analyzed for egg quality parameters. The other three eggs from each replicate were randomly selected and later analyzed for the content of water, crude protein, crude fat, and yolk cholesterol.

Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract. Calcium and potassium concentrations were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800 while phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer Lambda 45 (AOAC, 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were measured according to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg quality parameters were measured in all cases within 48 hours of collection.

Statistical analysis

Data generated were subjected to statistical analysis using one-way ANOVA (R Core Team, 2018) in a completely randomized design. Tukey test was used to separate significant treatment means, and significance was declared if $P < 0.05$.

Result and discussion

The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio (Data not shown). Egg, albumen, and yolk weight were also not different ($P > 0.05$) while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic treatments compared to the basal diet treatment (Table 2). The range of the eggshell weight was similar to the Sumiati et al. (2020) in egg of local Pajajaran duck (7.97 to 8.46 g) but higher than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained in the organic acid + probiotic supplementation; though the present study did not measure calcium and phosphorus content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphorus in the lower pH of the digestive tracts (Soltan, 2008). Supplementation of the mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability of calcium and phosphorus due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the minerals retention and bone mineralization (Ziaie et al., 2011).

The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score was higher than that in the egg of the Pajajaran duck (5.56 to 8.89) and could be categorized as good quality egg (range from 9.00 to 12.00) (Sudaryani, 2000). The highest mean of the colour index was found in the organic acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might related to a higher absorption of beta carotene. The yolk index of the duck in this study was lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al., 2012). Yolk index is mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index values were in line with the protein contents of the egg where the highest protein content was also found in the organic acid+probiotic treatment while the lowest in the basal diet.

The organic acid supplementation improved Haugh unit and the cholesterol content in the yolk ($P <$ 0.05) though the drinking water was already acidic. The lactic and acetic acid in the acid supplementation could improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the previous studies reported (Mohan et al., 1995; Abdulrahim et al., 1996).

The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and cholesterol content ($P < 0.01$). The range of Haugh unit score in the present work was similar to the range in the study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic acid+probiotic supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a result of biological and chemical activity produced by the organic acid+probiotic in the digestive tract. According to previous studies (Tomaro-Duchesneau et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liong (2010) reviewed that three mechanisms could cause the decrease in cholesterol and fat levels: 1) inhibition of cholesterol synthesis due to probiotic fermented compounds, 2) bile salt deconjugation, which causes a higher use of cholesterol for bile salt synthesis, and 3) the ability of probiotics to bind cholesterol. Furthermore, the decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study in Shaoxing duck also reported that supplementation of *Bacillus subtilis* reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L by (Li et al., 2011).

Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat, and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the highest yolk color index was found in organic acid+probiotic supplementation. The yolk fat and cholesterol content were lower than those in the other supplementations.

Acknowledgements The authors are grateful to Universitas Sriwijaya for the financial support of this research. We sincerely thank an anonymous reviewer for critically reading and suggesting substantial improvements.

Author contribution Original intellectual concept and study design: SS; Methodology: FY and MS; Data curation, formal analysis, and investigation: ES, BM and A; Writing - original draft preparation: AA; Writing - review and editing: MR and AA; Funding acquisition: SS. All authors read and approved the final manuscript.

Funding The research received funding from a competitive grant (number 096/SP2H/LT/DRPM/IV/2019).

Data availability on request.

Code availability not applicable

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Consent to participate not applicable

Consent for publication not applicable

Conflict of interest the authors declare that they have no competing interest.

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Table 1 Composition and nutrients content of the basal feed (dry matter basis)

Ingredients	**%**	**Nutrient**	**%**
Maize grain	43.00	Metabolizable energy	2771.08*
Rice bran	19.80	Crude protein	23.97
Comercial concentrate	19.10	Crude fibre	9.64
Soybean meal	12.20	Ether extract	5.91
Meat bone meal	4.90	Calcium	2.86
Methionine	0.40	Phosphorus	1.08
Lysine	0.60	Potassium	0.45

*Kcal/kg, calculated based on NRC (1994)

Table 2 Effect of feeding containing probiotic, organic acid, and tetracycline on egg quality of Pegagan ducks

Parameters	Experimental treatments						SEM	P value
	Basal	Basal + organic acid	Basal + probiotic	Basal + tetracycline	Basal + organic acid + probiotic	Basal + organic acid + tetracycline		
Egg weight (g)	59.10	60.12	62.56	61.81	63.24	61.40	0.562	0.291
Albumen weight (g)	31.15	31.78	33.9	32.71	34.85	32.44	0.526	0.365
Yolk weight (g)	17.41	17.68	18.79	17.89	19.11	18.48	0.355	0.749
Eggshell weight (g)	7.64^{b}	7.87^{ab}	8.83^{a}	8.53^{ab}	8.96^{a}	7.86^{ab}	0.166	0.035
Yolk color score	10.41^{b}	10.75^{b}	10.83^{b}	10.79^{b}	11.92^{a}	10.83^{b}	0.130	0.008
Egg index	71.23^{c}	72.97^{bc}	76.07^{b}	74.65^{bc}	79.36^{a}	73.19^{bc}	0.753	0.014
Albumen index	0.12	0.13	0.13	0.13	0.14	0.13	0.003	0.959
Yolk index	0.38	0.39	0.41	0.41	0.42	0.40	0.008	0.873
Haugh unit (HU)	88.80^{c}	90.10^{b}	92.34^{ab}	91.27^{ab}	92.44^{a}	91.10^{ab}	0.369	0.017
Water Content (%)	63.07	62.48	61.97	62.79	59.33	61.55	0.495	0.288
Protein (%)	12.25	13.11	13.56	12.73	14.02	12.99	0.201	0.149
Yolk Fat (%)	27.09^{a}	26.20^{ab}	26.09^{b}	26.21^{ab}	24.09^{c}	24.99^{bc}	0.253	0.001
Yolk Cholesterol (mg/dl)	465.26^{a}	327.76^{b}	313.99^{b}	348.01^{b}	284.75^{c}	306.62^{b}	14.260	0.000

SEM: Standard error of mean

Within rows, means with different superscripts differ at $P < .05$ (Tukey post hoc test).

Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck: a research note

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Abstract

The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. ~~It used~~ 72 Pegagan laying ducks~~,~~ (Average age: 24 weeks) ~~old which~~ were randomly allocated to six treatment groups: basal diet, basal diet+organic acid, basal diet+probiotic, basal diet+tetracycline, basal diet+probiotic+organic acid, and basal diet+organic acid+tetracycline. The result showed that the feeding diets containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk colour score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significantly ($P > 0.05$) different. It is concluded that dietary supplementation of organic acid and probiotic derived from grass silage improved egg quality in terms of yolk colour score, fat and cholesterol content ~~(11.92, 24.08%, and 284.75 mg/dl, respectively)~~.

Keyword Probiotics, Organic Acid Salt, Egg Quality, Pegagan Duck

Introduction

Probiotic suplementation has been found to improve growth performance and feed conversion ratio of broilers (Patel et al., 2015), egg size, egg mass, egg weight (Jin et al., 1997) and ~~and~~ reduce egg yolk cholesterol in laying hen (Mohan et al., 1995; Abdulrahim et al., 1996). The utilization of probiotics could improve the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal host by stimulating the synthesis of vitamins B-group, improving host immunity, and increasing volatile fatty acids.

Pancreatic secretion was increased by organic acids supplementation (Dibner and Buttin, 2002). Villus height in the small intestines and serum calcium and phosphorus concentrations were also elevated (Adil et al., 2010). Organic acids supplementation was associated with lowering the pH of the digestive tract and related to reductions of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke, 2003). Moreover, organic acids supplementation improved feed efficiency, egg mass, eggshell quality, and yolk index in layer hen (Soltan, 2008).

Lowland serves potential contributions for the farming sustainability in South Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic flooding. The Pegagan duck is considered more suitable for small-scale farmers that provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah, 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to the duck egg quality parameters have not been tested. When the digestive tract of the duck was presumably acidic, OoOrganic acids and lactic acid bacteria derived from a grass silage might serve as a valuable alternative of additive for an improvement of the digestive health and absorptionsince simple and less expensive preparation (Sandi et al., 2020). The organic acids could serve not only for lowering pH when the digestive tracts were already acidic. Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the effectiveness of supplements and antibiotic tetracycline.

Materials and Methods

Experimental animal and treatments

An experimental trial was conducted for 10 weeks at the experimental station, Faculty of Agriculture, Universitas Sriwijaya. Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate. The ducks were kept in experimental pens (1.5 m^2, ~~four~~ three birds in each pen) in an open-sided barn with 5 cm of rice husk litter after proper cleaning and disinfection. The ~~animals~~ birds were fed with a basal diet (NRC, 1994) that was processed as crumble (Table 1). The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.~~24 h.~~

Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2 %, v/v) was added in drinking water. The silage was prepared from a swamp grass (*Hymenachne acutigluma*) plus 3% molasses (w/w, fresh matter basis). An isolate from the silage was incubated in the deMann Rogosa Sharp medium (a selective medium for lactic acid bacteria) for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the supernatant and substrate. The substrate contained ~~had~~ 8.24 colony-forming unit~~/ml~~ of *Lactobacillus plantarum* per ml. The substrate was mixed with an amount of 5% (w/w) of the mixture of milk skim and maltodextrin and then dried at 37 ºC for 48 hours (Sandi et al., 2018). Organic acid was prepared by mixing the distilled water to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered and stored in the refrigerator (5 ºC). Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of acetic acid (v/v) (Sandi et al., 2020).

Laboratory analyses and measurement methods

Feed offered was sampled each week and then pooled at the end of the experiment. Seven fresh eggs from each replicate at the end of this study were randomly selected and later analyzed for egg quality parameters. The other three eggs from each replicate were randomly selected and later analyzed for the content of water, crude protein, crude fat, and yolk cholesterol.

Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract~~, calcium, phosphorus, and potassium according to the methods of AOAC (2005).~~ Calcium and potassium concentrations ~~of Ca and Mg~~ were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800 while ~~P~~phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer Lambda 45 (AOAC, 2005).

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Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were measured according to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg quality parameters were measured in all cases within 48 hours of collection.

Statistical analysis

Data generated were subjected to statistical analysis using one-way ANOVA (R Core Team, 2018) in a completely randomized design. Tukey test was used to separate significant treatment means, and significance was declared if $P < 0.05$.

Result and discussion

The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio (Data not shown). Egg, albumen, and yolk weight were also not different ($P > 0.05$) while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic treatments compared to the basal diet treatment (Table 2). The range of the eggshell weight was similar to the Sumiati et al. (2020) in egg of local Pajajaran duck (7.97 to 8.46 g) but higher than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained in the organic acid + probiotic supplementation; though the present study did not measure calcium and phosphorus content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphorus in the lower pH of the digestive tracts (Soltan, 2008). Supplementation of the mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability of calcium and phosphorus due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the minerals retention and bone mineralization (Ziaie et al., 2011).

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The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score was higher than that in the egg of the Pajajaran duck (5.56 to 8.89) and could be categorized as good quality egg (range from 9.00 to 12.00) (Sudaryani, 2000). The highest mean of the colour index was found in the organic acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might related to a higher absorption of beta carotene. The yolk index of the duck in this study was lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al., 2012). Yolk index is mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index values were in line with the protein contents of the egg where the highest protein content was also found in the organic acid+probiotic treatment while the lowest in the basal diet.

The organic acid supplementation improved Haugh unit and the cholesterol content in the yolk (*P* < 0.05) though the . It seems that the acid drinking water might have less effect to the pH of the digestive tract but this needs further investigation was already acidic. The lactic and acetic acid in the acid supplementation could improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the previous studies reported (Mohan et al., 1995; Abdulrahim et al., 1996).

The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and cholesterol content in the yolk (P < 0.01). The range of Haugh unit score in the present work was similar to the range in the study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic acid+probiotic supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a result of the biological and chemical activity produced by the organic acid+probiotic supplementation, where the accumulation of these two substrates causes changes in the lipid profile in the digestive systemtract. According to previous studies (Tomaro-Duchesneau et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liong (2010) reviewed that A study conducted to determine the effect of using probiotics on lowering cholesterol levels reported that tThere are 3 tTthree mechanisms that couldan cause athe decrease in cholesterol and fat levels: 1), namely 1). inhibition of cholesterol synthesis due to probiotic fermented compounds,; 2) . Bbile salt deconjugation, which causes a higher use of cholesterol for bile salt synthesis, and and results in lower cholesterol levels; 3) t. The existence of the ability of probiotics to bind cholesterol, causing low cholesterol levels . Furthermore, tThe decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study in Shaoxing duck also reported that supplementation of *Bacillus subtilis* reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L by (Li et al., 2011)was. The role of probiotics in reducing egg cholesterol could be related to the changing lipid metabolism in the digestive tract. According to previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the instestines for cell metabolism and thus reducing the total absorption of lipid.

Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat, and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the highest yolk color index was found in organic acid+probiotic supplementation. The yolk fat and cholesterol content were lower than those in the other supplementations.

Acknowledgements The authors are grateful to Universitas Sriwijaya for the financial support of this research. We sincerely thank an anonymous reviewer for critically reading and suggesting substantial improvements.

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Author contribution Original intellectual concept and study design: SS; Methodology: FY and MS; Data curation, formal analysis, and investigation: ES, BM and A; Writing - original draft preparation: AA; Writing - review and editing: MR and AA; Funding acquisition: SS. All authors read and approved the final manuscript.

Funding The research received funding from a competitive grant (number 096/SP2H/LT/DRPM/IV/2019).

Data availability on request.

Code availability not applicable

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

Consent to participate not applicable

Consent for publication not applicable

Conflict of interest the authors declare that they have no competing interest.

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Table 1 Composition and nutrients content of the basal feed (dry matter basis)

Ingredients	**%**	**Nutrient**	**%**
Maize grain	43.00	Metabolizable energy	2771.08*
Rice bran	19.80	Crude protein	23.97
Comercial concentrate	19.10	Crude fibre	9.64
Soybean meal	12.20	Ether extract	5.91
Meat bone meal	4.90	Calcium	2.86
Methionine	0.40	Phosphorus	1.08
Lysine	0.60	Potassium	0.45

*Kcal/kg, calculated based on NRC (1994)

Table 2 Effect of feeding containing probiotic, organic acid, and tetracycline on egg quality of Pegagan ducks

Parameters	Experimental treatments						SEM	*P* value
	Basal	Basal + organic acid	Basal + probiotic	Basal + tetracycline	Basal + organic acid + probiotic	Basal + organic acid + tetracycline		
Egg weight (g)	59.10	60.12	62.56	61.81	63.24	61.40	0.562	0.291
Albumen weight (g)	31.15	31.78	33.9	32.71	34.85	32.44	0.526	0.365
Yolk weight (g)	17.41	17.68	18.79	17.89	19.11	18.48	0.355	0.749
Eggshell weight (g)	7.64[b]	7.87[ab]	8.83[a]	8.53[ab]	8.96[a]	7.86[ab]	0.166	0.035
Yolk color score	10.41[b]	10.75[b]	10.83[b]	10.79[b]	11.92[a]	10.83[b]	0.130	0.008
Egg index	71.23[c]	72.97[bc]	76.07[b]	74.65[bc]	79.36[a]	73.19[bc]	0.753	0.014
Albumen index	0.12	0.13	0.13	0.13	0.14	0.13	0.003	0.959
Yolk index	0.38	0.39	0.41	0.41	0.42	0.40	0.008	0.873
Haugh unit (HU)	88.80[c]	90.10[b]	92.34[ab]	91.27[ab]	92.44[a]	91.10[ab]	0.369	0.017
Water Content (%)	63.07	62.48	61.97	62.79	59.33	61.55	0.495	0.288
Protein (%)	12.25	13.11	13.56	12.73	14.02	12.99	0.201	0.149
Yolk Fat (%)	27.09[a]	26.20[ab]	26.09[b]	26.21[ab]	24.09[c]	24.99[bc]	0.253	0.001
Yolk Cholesterol (mg/dl)	465.26[a]	327.76[b]	313.99[b]	348.01[b]	284.75[c]	306.62[b]	14.260	0.000

SEM: Standard error of mean

Within rows, means with different superscripts differ at $P < .05$ (Tukey post hoc test).

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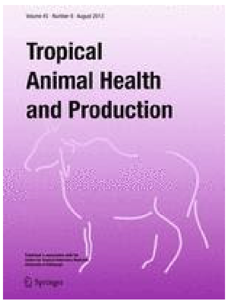

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Schedule	Received Revised Accepted	28 Apr 2021 4 Jan 2022
Abstract	The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. Seventy-two Pegagan laying ducks (average age: 24 weeks) were randomly allocated to six treatment groups: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + probiotic + organic acid, and basal diet + organic acid + tetracycline. The result showed that the feeding diets containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk color score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significantly ($P > 0.05$) different. It is concluded that dietary supplementation of organic acid and probiotic derived from grass silage improved egg quality in terms of yolk color score, fat, and cholesterol content.	
Keywords (separated by '-')	Probiotics - Organic acid salt - Egg quality - Pegagan duck	
Footnote Information		

Tropical Animal Health and Production _####################_
https://doi.org/10.1007/s11250-022-03060-7

SHORT COMMUNICATIONS

Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck: a research note

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Received: 28 April 2021 / Accepted: 4 January 2022

Abstract

The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. Seventy-two Pegagan laying ducks (average age: 24 weeks) were randomly allocated to six treatment groups: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + probiotic + organic acid, and basal diet + organic acid + tetracycline. The result showed that the feeding diets containing probiotics and organic acid significantly ($P < 0.05$) reduced yolk fat and yolk cholesterol and increased eggshell weight, egg index, yolk color score, Haugh unit, and protein content. However, egg weight, albumen weight, yolk weight, albumen index, yolk index, and water content were not significantly ($P > 0.05$) different. It is concluded that dietary supplementation of organic acid and probiotic derived from grass silage improved egg quality in terms of yolk color score, fat, and cholesterol content.

Keywords Probiotics · Organic acid salt · Egg quality · Pegagan duck

Introduction

Probiotic supplementation has been found to improve growth performance and feed conversion ratio of broilers (Patel et al. 2015), egg size, egg mass, and egg weight (Jin et al. 1997) and reduce egg yolk cholesterol in laying hens (Mohan et al. 1995; Abdulrahim et al. 1996). The utilization of probiotics could improve the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal by stimulating the synthesis of vitamin B group, improving immunity, and increasing volatile fatty acids.

Pancreatic secretion was increased by organic acid supplementation (Dibner and Buttin 2002). Villus height in the small intestines and serum calcium and phosphorus concentrations were also elevated (Adil et al. 2010). Organic acid supplementation was associated with lowering the pH of the digestive tract and related to reduction of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella*, and *Campylobacter*. The lower pH was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin 2002; Ricke, 2003). Moreover, organic acid supplementation improved feed efficiency, egg mass, eggshell quality, and yolk index in layer hen (Soltan 2008).

Lowland serves potential contributions for the farming sustainability in South Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic flooding. The Pegagan duck is considered more suitable for small-scale farmers that provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy 2020).

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Journal : Large 11250 | Article No : 3060 | Pages : 5 | MS Code : 3060 | Dispatch : 14-1-2022

To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to the duck egg quality parameters have not been tested. Organic acids and lactic acid bacteria derived from grass silage might serve as a valuable alternative of additive since it is simple and has less expensive preparation (Sandi et al. 2020). The organic acids could serve not only for lowering pH when the digestive tracts were already acidic. Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the effectiveness of supplements and antibiotic tetracycline.

Materials and methods

Experimental animal and treatments

An experimental trial was conducted for 10 weeks at the experimental station, Faculty of Agriculture, Universitas Sriwijaya. Laying Pegagan ducks (*n*: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate. The ducks were kept in experimental pens (1.5 m^2, three birds in each pen) in an open-sided barn with 5 cm of rice husk litter after proper cleaning and disinfection. The birds were fed with a basal diet (NRC 1994) that was processed as crumble (Table 1). The treatments were as follows: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.

Probiotic and tetracycline (0.2%, dry matter basis) were mixed in the basal diet while organic acid (0.2%, v/v) was added to drinking water. The silage was prepared from a swamp grass (*Hymenachne acutigluma*) plus 3% molasses (w/w, fresh matter basis). An isolate from the silage was incubated in the deMann Rogosa Sharp medium (a selective medium for lactic acid bacteria) for 48 h. The culture was centrifuged at 3000 rpm for 15 min to separate the supernatant and substrate. The substrate contained 8.24 colony-forming units of *Lactobacillus plantarum* per ml. The substrate was mixed with an amount of 5% (w/w) of the mixture of milk skim and maltodextrin and then dried at 37 °C for 48 h (Sandi et al. 2018). Organic acid was prepared by mixing the distilled water to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered and stored in the refrigerator (5 °C). Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of acetic acid (v/v) (Sandi et al. 2020).

Table 1 Composition and nutrients content of the basal feed (dry matter basis)

Ingredients	%	Nutrient	%
Maize grain	43.00	Metabolizable energy	2771.08*
Rice bran	19.80	Crude protein	23.97
Comercial concentrate	19.10	Crude fiber	9.64
Soybean meal	12.20	Ether extract	5.91
Meat bone meal	4.90	Calcium	2.86
Methionine	0.40	Phosphorus	1.08
Lysine	0.60	Potassium	0.45

*Kcal/kg, calculated based onNRC (1994)

Laboratory analyses and measurement methods

Feed offered was sampled each week and then pooled at the end of the experiment. Seven fresh eggs from each replicate at the end of this study were randomly selected and later analyzed for egg quality parameters. The other three eggs from each replicate were randomly selected and later analyzed for the content of water, crude protein, crude fat, and yolk cholesterol.

Samples of the basal diet were analyzed for dry matter, crude protein, crude fiber, and extract. Calcium and potassium concentrations were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800 while phosphorus concentration was analyzed using a spectrophotometer Perkin Elmer Lambda 45 (AOAC 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance (Ohaus CP214). Yolk color score, egg index, yolk index, albumen index, and Haugh unit were measured according to Card and Nesheim (1972) while cholesterol content was analyzed according to Diplock et al. (1991). Egg quality parameters were measured in all cases within 48 h of collection.

Statistical analysis

Data generated were subjected to statistical analysis using one-way ANOVA (R Core Team 2018) in a completely randomized design. Tukey's test was used to separate significant treatment means, and significance was declared if $P < 0.05$.

Result and discussion

The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio (data not shown). Egg, albumen, and yolk weight were also not different ($P > 0.05$) while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid + probiotic treatments compared to the basal diet treatment (Table 2). The

Table 2 Effect of feeding containing probiotic, organic acid, and tetracycline on egg quality of Pegagan ducks

Parameters	Experimental treatments						SEM	*P* value
	Basal	Basal + organic acid	Basal + probiotic	Basal + tetracycline	Basal + organic acid + probiotic	Basal + organic acid + tetracycline		
Egg weight (g)	59.10	60.12	62.56	61.81	63.24	61.40	0.562	0.291
Albumen weight (g)	31.15	31.78	33.9	32.71	34.85	32.44	0.526	0.365
Yolk weight (g)	17.41	17.68	18.79	17.89	19.11	18.48	0.355	0.749
Eggshell weight (g)	7.64^{b}	7.87^{ab}	8.83^{a}	8.53^{ab}	8.96^{a}	7.86^{ab}	0.166	0.035
Yolk color score	10.41^{b}	10.75^{b}	10.83^{b}	10.79^{b}	11.92^{a}	10.83^{b}	0.130	0.008
Egg index	71.23^{c}	72.97^{bc}	76.07^{b}	74.65^{bc}	79.36^{a}	73.19^{bc}	0.753	0.014
Albumen index	0.12	0.13	0.13	0.13	0.14	0.13	0.003	0.959
Yolk index	0.38	0.39	0.41	0.41	0.42	0.40	0.008	0.873
Haugh unit (HU)	88.80^{c}	90.10^{b}	92.34^{ab}	91.27^{ab}	92.44^{a}	91.10^{ab}	0.369	0.017
Water content (%)	63.07	62.48	61.97	62.79	59.33	61.55	0.495	0.288
Protein (%)	12.25	13.11	13.56	12.73	14.02	12.99	0.201	0.149
Yolk fat (%)	27.09^{a}	26.20^{ab}	26.09^{b}	26.21^{ab}	24.09^{c}	24.99^{bc}	0.253	0.001
Yolk cholesterol (mg/dl)	465.26^{a}	327.76^{b}	313.99^{b}	348.01^{b}	284.75^{c}	306.62^{b}	14.260	0.000

SEM standard error of mean

Within rows, means with different superscripts differ at $P < .05$ (Tukey's post hoc test)

range of the eggshell weight was similar to the Sumiati et al. (2020) in the egg of local Pajajaran duck (7.97 to 8.46 g) but higher than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained in the organic acid + probiotic supplementation; though the present study did not measure calcium and phosphorus content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphorus in the lower pH of the digestive tracts (Soltan 2008). Supplementation of the mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability of calcium and phosphorus due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the mineral retention and bone mineralization (Ziaie et al. 2011).

The yolk color was affected ($P < 0.01$) by the treatments (Table 2). The yolk color score was higher than that in the egg of the Pajajaran duck (5.56 to 8.89) and could be categorized as a good quality egg (range from 9.00 to 12.00) (Sudaryani 2000). The highest mean of the color index was found in the organic acid + probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might be related to a higher absorption of beta carotene. The yolk index of the duck in this study was lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al. 2012). Yolk index is mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer of water between yolk and albumen (Bell 2002). The yolk index values were in line with the protein contents of the egg where the highest protein content was also found in the organic acid + probiotic treatment while the lowest in the basal diet.

The organic acid supplementation improved the Haugh unit and the cholesterol content in the yolk ($P < 0.05$) though the drinking water was already acidic. The lactic and acetic acid in the acid supplementation could improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the previous studies reported (Mohan et al. 1995; Abdulrahim et al. 1996).

The organic acid + probiotic supplementation improved the Haugh unit of the egg and decreased fat and cholesterol content ($P < 0.01$). The range of Haugh unit score in the present work was similar to the range in the study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic acid + probiotic supplementation (Table 2). The decrease in fat and cholesterol in the yolk with organic acid + probiotics is presumably due to a reduction of serum and plasma cholesterol contents (Adil et al. 2010) as a result of biological and chemical activity produced by the organic acid + probiotic in the digestive tract. According to previous studies (Tomaro-Duchesneau et al. 2014; Park et al. 2018), probiotics could assimilate the cholesterol in the intestines for cell metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liong (2010) reviewed that three mechanisms could cause the decrease in cholesterol and fat levels: (1) inhibition of cholesterol synthesis due to probiotic fermented compounds, (2) bile salt deconjugation, which causes a higher use of cholesterol for bile salt synthesis, and (3) the ability of probiotics to bind cholesterol. Furthermore, the decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al. 1995; Abdulrahim et al. 1996; Jin et al. 1997). A study in Shaoxing duck also reported that supplementation of *Bacillus subtilis* reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L (Li et al. 2011).

Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat, and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the highest yolk color index was found in organic acid + probiotic supplementation. The yolk fat and cholesterol content were lower than those in the other supplementations.

Acknowledgements The authors are grateful to Universitas Sriwijaya for the financial support of this research. We sincerely thank an anonymous reviewer for critically reading and suggesting substantial improvements.

Author contribution Original intellectual concept and study design: SS; methodology: FY and MS; data curation, formal analysis, and investigation: ES, BM, and A; writing—original draft preparation: AA; writing—review and editing: MR and AA; funding acquisition: SS. All authors read and approved the final manuscript.

Funding The research received funding from a competitive grant (number 096/SP2H/LT/DRPM/IV/2019).

Data availability On request.

Code availability Not applicable.

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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