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

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

2

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12

13 **Abstract**

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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).

24

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

25

26 **Introduction**

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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 supplementation 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;

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

37 Organic acids have been used for a long time as feed additives and commonly referred to as acidifiers or
38 acidifying agents that have shown favorable effects on the nutrient utilization. Dietary supplementation with
39 organic acids was associated with lowering the pH of the digestive tract and related to reductions of acid-intolerant
40 bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH was also related to an increase
41 of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke, 2003). The supplementation of
42 organic acids also improved feed efficiency, egg mass, egg shell quality, and yolk index in layer hen (Soltan,
43 2008).

44 Lowland farming serves potential contributions for the sustainability of food production in South
45 Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic
46 flooding during the rainy season. The Pegagan duck is considered more suitable for small-scale farmers in the
47 region. The waterfowl provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah,
48 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards
49 in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol
50 in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of
51 cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

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

56

57 **Materials and Methods**

58 **Study site**

59 An animal feeding experiment was conducted for 10 weeks at the experimental station, Department of
60 Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The ducks were cared for according to the Animal

61 Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from
62 Universitas Sriwijaya.

63

64 **Experimental animal and treatments**

65 Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed
66 and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate.
67 The ducks were kept in experimental pens (1.5 m², four birds in each pen) with 5 cm of rice husk litter. The pens
68 were located in an open-sided barn after proper cleaning and disinfection. The animals were fed with a basal diet
69 (NRC, 1994) that was processed as crumble. The composition and nutrient concentrations of the basal diet are
70 shown in Table 1. The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet +
71 tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water
72 were provided ad libitum while the lighting was provided 24 h.

73 Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2
74 %, v/v) was added in drinking water. The probiotic preparation was referred to the method of Bregni et al. (2000).
75 The silage was prepared from a local swamp grass (Kumpai Tembaga grass, *Hymenachne acutigluma*) plus 3%
76 molasses (w/w, fresh matter basis) (Sandi et al., 2018). An isolate from the silage was incubated in the deMann
77 Rogosa Sharp medium for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the
78 supernatant and substrate. An amount of 5% (w/w) of the mixture of milk skim and maltodextrin was added to
79 the substrate. Afterward, the substrate was dried at 37 °C for 48 hours. Organic acid preparation was referred to
80 Negara (2009): The distilled water was added to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then
81 filtered and stored in the refrigerator (5 °C) before use.

82

83 **Sampling and handling**

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

89 **Laboratory analyses and measurement methods**

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

96 **Statistical analysis**

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

101

102 **Result and discussion**

103 The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-egg ratio
104 (Data not shown). Egg weight, albumen weight, and yolk weight were also not different ($P > 0.05$) among the
105 treatments while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic treatments
106 compare to the basal diet treatment (Table 2). The range of the eggshell weight in the present study was similar
107 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
108 range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was obtained
109 in the organic acid + probiotic supplementation. Though the present study did not measure calcium and phosphor
110 content in the eggshell, this could be related to the higher digestion and absorption of calcium and phosphor in
111 the lower pH of the digestive tracts (Soltan, 2008; Abbas et al., 2013). Supplementation of the mixture of organic
112 acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the digestibility and availability
113 of calcium and phosphor due to developing desirable microflora (*Lactobacillus* sp) that results in increasing the
114 minerals retention and bone mineralization (Ziaie et al., 2011).

115 The yolk color was affected ($P < 0.01$) by the treatments (Table 2). The yolk color score ranged from 10.41 to
116 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
117 be categorized as good quality egg according to Sudaryani (2000) where the good color score range from 9.00 to
118 12.00. The highest mean of the color index was found in the organic acid+probiotic treatment. The improvement

119 of nutrient absorption due to the combination of the organic and probiotic supplementation might relate to a higher
120 absorption of beta carotene. The yolk index of the duck in this study ranged from 0.38 to 0.41. The range was
121 lower than the range in the egg of Muscovy duck (0.40 to 0.41) (Etuk et al., 2012). Yolk index is mainly influenced
122 by the protein content of diet which can stimulate the formation of membrane vitellin that regulates the transfer
123 of water between yolk and albumen (Bell, 2002). The yolk index value was in line with the protein contents of
124 the egg in the present study where the highest protein content was also found in the organic acid+probiotic
125 treatment while the lowest in the basal diet.

126 The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and
127 cholesterol content in the yolk ($P < 0.01$). The range of Haugh unit score in the present work was similar to the
128 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
129 Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study.
130 The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic
131 acid+probiotic supplementation (Table 2). The decreased fat and cholesterol content were consistent with the
132 previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study by Li
133 et al. (2011) was also reported that supplementation of *Bacillus subtilis* in the diet of Shaoxing duck reduced the
134 yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L. The role of probiotics in reducing egg cholesterol
135 could be related to the changing lipid metabolism in the digestive tract. According to previous studies (Catherine
136 et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the intestines for cell metabolism
137 and thus reducing the total absorption of lipid.

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

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144 Meisji L. Sari; Data curation, formal analysis, and investigation: Eli Sahara, Bella P. Maharani, Asmak; Writing
145 - original draft preparation: Asep I. M. Ali; Writing - review and editing: Muhamad N. Rofiq, Asep I. M. Ali;
146 Funding acquisition: Sofia Sandi. All authors read and approved the final manuscript.

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148 **Data availability** on request.

149 **Code availability** not applicable

150 **Declarations**

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

153 **Consent to participate** not applicable

154 **Consent for publication** not applicable

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

156 **References**

157 Abbas, G., Khan, S.H. and Rehman, H., 2013. Effects of formic acid administration in the drinking water on
158 production performance, egg quality and immune system in layers during hot season. *Avian Biology*
159 *Research*, 6, 227–232.

160 Abdelqader, A., Irshaid, R. and Al-Fataftah, A.-R., 2013. Effects of dietary probiotic inclusion on performance,
161 eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production.
162 *Tropical Animal Health and Production*, 45, 1017–1024.

163 Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K., 1996. The influence of
164 *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma
165 and egg yolk. *British Poultry Science*, 37, 341–346.

166 AOAC, 2005. *Official Methods of Analysis of AOAC International*. Official Methods of Analysis, (AOAC:
167 Gaithersburg, MD).

168 Bell D. 2002. Formation of the egg. In: Bell DD, Weaver WD, editors. *Commercial chicken meat and egg*
169 *production 5th edition volume II*, (Springer Science: New York).

170 Bregni, C., Degrossi, J., Garcia, R., Lamas, M.C., Firenstein, R., D'aquino, M. and others, 2000. Alginate
171 microspheres of *Bacillus subtilis*. *Ars Pharmaceutica*, 41, 245–248

172 Card, L.E. and Nesheim, M., 1972. *Poultry Production*, (Lea and febiger Press: Philadelphia).

173 Catherine, T.D., Jones, M.L., Shah, D., Jain, P., Saha, S. and Prakash, S., 2014. Cholesterol assimilation by
174 *Lactobacillus* probiotic bacteria: An in vitro investigation. *BioMed Research International*, 2014, 1–9.

175 Danahy, A., 2020. Duck eggs: Nutrition, benefits, and side effects. [https://www.healthline.com/nutrition/duck-](https://www.healthline.com/nutrition/duck-eggs#nutrition)
176 [eggs#nutrition](https://www.healthline.com/nutrition/duck-eggs#nutrition). Accessed 19 April 2020.

177 Dibner, J.J. and Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition
178 and metabolism. *Journal of Applied Poultry Research*, 11, 453–463.

179 Diplock, A.T., Symons, M.C.R. and Rice-Evans, C.A., 1991. Techniques in free radical research, (Elsevier
180 Science).

181 Etuk, I.F., Ojewola, G.S., Abasiokong, S.F., Amaefule, K.U. and Etuk, E.B., 2012. Egg quality of Muscovy ducks
182 reared under different management systems in the humid tropics. *Revista Científica UDO Agrícola*, 12,
183 226–229.

184 Fouad, A.M., Ruan, D., Lin, Y.C., Zheng, C.T., Zhang, H.X., Chen, W., Wang, S., Xia, W.G. and Li, Y., 2016.
185 Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying ducks.
186 *British Poultry Science*, 57, 818–823.

187 Fuller, M.F. and Cadenhead, A., 1991. Effect of the amount and composition of the diet on galactosamine flow
188 from the small intestine. (EAAP: April 24 - 26. Wageningen, Netherlands. p 330).

189 Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S., 1997. Probiotics in poultry: modes of action. *World's Poultry
190 Science Journal*, 53, 351–368.

191 Li, W.F., Rajput, I.R., Xu, X., Li, Y.L., Lei, J., Huang, Q. and Wang, M.Q., 2011. Effects of probiotic (*Bacillus
192 subtilis*) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck.
193 *International Journal of Poultry Science*, 10, 583–589.

194 Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A., 1995. Effect of probiotic supplementation on
195 serum/yolk cholesterol and on egg shell thickness in layers. *British Poultry Science*, 36, 799–803.

196 Negara, W. 2009. Study of Organic Acid Salt Production from Complete Ration Silage as a Growth Booster in
197 Broilers. M.Sc Thesis. IPB. Bogor. Indonesia.

198 NRC, 1994. Nutrient requirements of poultry. National Research Council, (National Academy Press: Washington
199 D.C)

200 Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y.G., Kim, A.R., Holzapfel, W. and Ji, Y., 2018.
201 Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome
202 and propionate level in a murine model. *PLoS ONE*, 13.

203 Patel, S.G., Raval, A.P., Bhagwat, S.R., Sadrasaniya, D.A., Patel, A.P. and Joshi, S.S., 2015. Effects of probiotics
204 supplementation on growth performance, feed conversion ratio and economics of broilers. *Journal of
205 Animal Research*, 5, 155.

206 Pelicano, E., Souza, P. de, Souza, H. de, Oba, A., Norkus, E., Kodawara, L. and Lima, T. de, 2003. Effect of
207 different probiotics on broiler carcass and meat quality. *Revista Brasileira de Ciência Avícola*, 5, 207–214.

208 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical

209 Computing. (Vienna, Austria).

210 Ricke, S., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry
211 Science, 82, 632–639.

212 Rolfe, R.D., 2000. The role of probiotic cultures in the control of gastrointestinal health. The Journal of Nutrition,
213 130, 396S-402S.

214 Sandi, S., Yosi, F., Sari, M.L. and Gofar, N., 2018. The characteristics and potential of lactic acid bacteria as
215 probiotics in silage made from *Hymenachne acutigluma* and *Neptunia oleracea* Lour. In: E3S Web of
216 Conferences.

217 Soltan, M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood
218 serum parameters in laying hens. International Journal of Poultry Sciences, 7, 613–621.

219 Sudaryani, T., 2000. Kualitas telur, (Penebar Swadaya: Jakarta).

220 Sumiati, S., Darmawan, A. and Hermana, W., 2020. Performances and egg quality of laying ducks fed diets
221 containing cassava (*Manihot esculenta* Crantz) leaf meal and golden snail (*Pomacea canaliculata*). Tropical
222 Animal Science Journal, 43, 227–232 .

223 Zahri, I. and Febriansyah, A., 2014. Business diversification and its impact on household income of farmers in
224 swampy lowland areas. Agricultural Socio-Economics Journal, 14, 144–153.

225 Ziaie, H., Bashtani, M., Torshizi, M.A.K., Naeemipour, H., Farhangfar, H., Zeinali, A. and others, 2011. Effect
226 of antibiotic and its alternatives on morphometric characteristics, mineral content and bone strength of tibia
227 in Ross broiler chickens. Global Veterinaria, 7, 315–322.

228

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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30 Juni 2021 pukul 01.26

Dear Dr. ali:

We have received the reports from our advisors on your manuscript, "Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck", which you submitted to Tropical Animal Health and Production.

Based on the advice received, your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions.

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

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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	

31 the performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful
32 microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal by
33 stimulating the synthesis of vitamins B-group, improving immunity, and increasing volatile fatty acids. The
34 improvement in digestive tracts resulted in an increase in intake and nutrient digestibility (Fuller and Cadenhead,
35 1991; Jin et al., 1997; Rolfe, 2000).

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

44 Lowland farming serves potential contributions for the sustainability of food production in South
45 Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic
46 flooding during the rainy season. The Pegagan duck is considered more suitable for small-scale farmers in the
47 region. The waterfowl provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah,
48 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards
49 in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol
50 in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of
51 cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

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

59

60 **Materials and Methods**

61 **Study site**

62 An animal feeding experiment was conducted for 10 weeks at the experimental station, Department of
63 Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The ducks were cared for according to the Animal
64 Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from
65 Universitas Sriwijaya.

66

67 **Experimental animal and treatments**

68 Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed
69 and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate.
70 The ducks were kept in experimental pens (1.5 m², three birds in each pen) with 5 cm of rice husk litter. The pens
71 were located in an open-sided barn after proper cleaning and disinfection. The birds were fed with a basal diet
72 (NRC, 1994) that was processed as crumble. The composition and nutrient concentrations of the basal diet are
73 shown in Table 1. The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal diet +
74 tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and water
75 were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.

76 Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2
77 %, v/v) was added in drinking water. The probiotic preparation was referred to the method of Bregni et al. (2000).
78 The silage was prepared from a local swamp grass (Kumpai Tembaga grass, *Hymenachne acutigluma*) plus 3%
79 molasses (w/w, fresh matter basis) (Sandi et al., 2018). An isolate from the silage was incubated in the deMann
80 Rogosa Sharp medium for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the
81 supernatant and substrate. The substrate contained 8.24 colony-forming unit of lactic acid bacteria per ml. An
82 amount of 5% (w/w) of the mixture of milk skim and maltodextrin was added to the substrate. Afterward, the
83 substrate was dried at 37 °C for 48 hours. Organic acid preparation was referred to Negara (2009): The distilled
84 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
85 refrigerator (5 °C) before use. Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of
86 acetic acid (v/v).

87

88 **Sampling and handling**

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

94 **Laboratory analyses and measurement methods**

95 Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract ether (AOAC,
96 2005). Calcium and potassium concentrations were analyzed using an atomic absorption spectrophotometer
97 Shimadzu AA-6800 while phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer
98 Lambda 45 (AOAC, 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an
99 electronic balance (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were
100 measured according to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et
101 al. (1991). Egg quality parameters were measured in all cases within 48 hours of collection.

102 **Statistical analysis**

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

107

108 **Result and discussion**

109 The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-
110 egg ratio (Data not shown). Egg weight, albumen weight, and yolk weight were also not different ($P > 0.05$)
111 among the treatments while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic
112 treatments compared to the basal diet treatment (Table 2). The range of the eggshell weight in the present study
113 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
114 than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was
115 obtained in the organic acid + probiotic supplementation. Though the present study did not measure calcium and
116 phosphorus content in the eggshell, this could be related to the higher digestion and absorption of calcium and
117 phosphorus in the lower pH of the digestive tracts (Soltan, 2008; Abbas et al., 2013). Supplementation of the

118 mixture of organic acid (propionic acid and sodium bentonite) in the broiler diet caused an increase in the
119 digestibility and availability of calcium and phosphorus due to developing desirable microflora (*Lactobacillus* sp)
120 that results in increasing the minerals retention and bone mineralization (Ziaie et al., 2011).

121 The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score ranged from
122 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
123 work could be categorized as good quality egg according to Sudaryani (2000) where the good color score range
124 from 9.00 to 12.00. The highest mean of the colour index was found in the organic acid+probiotic treatment. The
125 improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might
126 related to a higher absorption of beta carotene. The yolk index of the duck in this study ranged from 0.38 to 0.41.
127 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
128 mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that
129 regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index value was in line with the
130 protein contents of the egg in the present study where the highest protein content was also found in the organic
131 acid+probiotic treatment while the lowest in the basal diet.

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

136 The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and
137 cholesterol content ($P < 0.01$). The range of Haugh unit score in the present work was similar to the range in the
138 study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks
139 (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest Haugh
140 unit score was in line with the highest value of the egg weight and albumen weight in organic acid+probiotic
141 supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic acid+probiotics presumably
142 due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a result of biological and
143 chemical activity produced by the organic acid+probiotic in the digestive tract. According to previous studies
144 (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the intestines for cell
145 metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liang, (2010) reviewed that three
146 mechanisms could cause the decrease in cholesterol and fat levels: 1) inhibition of cholesterol synthesis due to
147 probiotic fermented compounds, 2) bile salt deconjugation, which causes a higher use of cholesterol for bile salt

148 synthesis, and 3) the ability of probiotics to bind cholesterol. Furthermore, the decreased fat and cholesterol
149 content were consistent with the previous study using layer chickens (Mohan et al., 1995; Abdulrahim et al.,
150 1996; Jin et al., 1997). A study by Li et al. (2011) also reported that supplementation of *Bacillus subtilis* in the
151 diet of Shaoxing duck reduced the yolk cholesterol content of eggs from 126.96 to 97.09 mmol/L.

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

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

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163 **Data availability** on request.

164 **Code availability** not applicable

165 **Declarations**

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

168 **Consent to participate** not applicable

169 **Consent for publication** not applicable

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

171 **References**

172 Abbas, G., Khan, S.H. and Rehman, H., 2013. Effects of formic acid administration in the drinking water on
173 production performance, egg quality and immune system in layers during hot season. *Avian Biology*
174 *Research*, 6, 227–232.

175 Abdelqader, A., Irshaid, R. and Al-Fataftah, A.-R., 2013. Effects of dietary probiotic inclusion on performance,
176 eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production.
177 *Tropical Animal Health and Production*, 45, 1017–1024.

178 Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K., 1996. The influence of
179 *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of
180 plasma and egg yolk. *British Poultry Science*, 37, 341–346.

181 Adil, S., Banday, T., Bhat, G.A., Mir, M.S. and Rehman, M., 2010. Effect of dietary supplementation of organic
182 acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary
183 Medicine International*, 2010, 479485

184 AOAC, 2005. *Official Methods of Analysis of AOAC International*. Official Methods of Analysis, (AOAC:
185 Gaithersburg, MD).

186 Bell D. 2002. Formation of the egg. In: Bell DD, Weaver WD, editors. *Commercial chicken meat and egg
187 production 5th edition volume II*, (Springer Science: New York).

188 Bregni, C., Degrossi, J., Garcia, R., Lamas, M.C., Firenstein, R., D'aquino, M. and others, 2000. Alginate
189 microspheres of *Bacillus subtilis*. *Ars Pharmaceutica*, 41, 245–248

190 Card, L.E. and Nesheim, M., 1972. *Poultry Production*, (Lea and febiger Press: Phidelphia).

191 Catherine, T.D., Jones, M.L., Shah, D., Jain, P., Saha, S. and Prakash, S., 2014. Cholesterol assimilation by
192 *Lactobacillus* probiotic bacteria: An in vitro investigation. *BioMed Research International*, 2014, 1–9.

193 Danahy, A., 2020. Duck eggs: Nutrition, benefits, and side effects. [https://www.healthline.com/nutrition/duck-](https://www.healthline.com/nutrition/duck-eggs#nutrition)
194 [eggs#nutrition](https://www.healthline.com/nutrition/duck-eggs#nutrition). Accessed 19 April 2020.

195 Dibner, J.J. and Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition
196 and metabolism. *Journal of Applied Poultry Research*, 11, 453–463.

197 Diplock, A.T., Symons, M.C.R. and Rice-Evans, C.A., 1991. *Techniques in free radical research*, (Elsevier
198 Science).

199 Etuk, I.F., Ojewola, G.S., Abasiokong, S.F., Amaefule, K.U. and Etuk, E.B., 2012. Egg quality of Muscovy ducks
200 reared under different management systems in the humid tropics. *Revista Científica UDO Agrícola*, 12,
201 226–229.

202 Fouad, A.M., Ruan, D., Lin, Y.C., Zheng, C.T., Zhang, H.X., Chen, W., Wang, S., Xia, W.G. and Li, Y., 2016.
203 Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying ducks.
204 *British Poultry Science*, 57, 818–823.

205 Fuller, M.F. and Cadenhead, A., 1991. Effect of the amount and composition of the diet on galactosamine flow
206 from the small intestine. (EAAP: April 24 - 26. Wageningen, Netherlands. p 330).

207 Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S., 1997. Probiotics in poultry: modes of action. *World's Poultry*

208 Science Journal, 53, 351–368.

209 Li, W.F., Rajput, I.R., Xu, X., Li, Y.L., Lei, J., Huang, Q. and Wang, M.Q., 2011. Effects of probiotic (*Bacillus*
210 *subtilis*) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck.
211 International Journal of Poultry Science, 10, 583–589.

212 Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A., 1995. Effect of probiotic supplementation on
213 serum/yolk cholesterol and on egg shell thickness in layers. British Poultry Science, 36, 799–803.

214 Negara, W. 2009. Study of Organic Acid Salt Production from Complete Ration Silage as a Growth Booster in
215 Broilers. M.Sc Thesis. IPB. Bogor. Indonesia.

216 NRC, 1994. Nutrient requirements of poultry. National Research Council, (National Academy Press: Washington
217 D.C)

218 Ooi, L.-G. and Liong, M.-T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo
219 and in vitro findings. International Journal of Molecular Sciences, 11, 2499–2522

220 Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y.G., Kim, A.R., Holzapfel, W. and Ji, Y., 2018.
221 Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome
222 and propionate level in a murine model. PLoS ONE, 13.

223 Patel, S.G., Raval, A.P., Bhagwat, S.R., Sadrasaniya, D.A., Patel, A.P. and Joshi, S.S., 2015. Effects of probiotics
224 supplementation on growth performance, feed conversion ratio and economics of broilers. Journal of
225 Animal Research, 5, 155.

226 Pelicano, E., Souza, P. de, Souza, H. de, Oba, A., Norkus, E., Kodawara, L. and Lima, T. de, 2003. Effect of
227 different probiotics on broiler carcass and meat quality. Revista Brasileira de Ciência Avícola, 5, 207–214.

228 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical
229 Computing. (Vienna, Austria).

230 Ricke, S., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry
231 Science, 82, 632–639.

232 Rolfe, R.D., 2000. The role of probiotic cultures in the control of gastrointestinal health. The Journal of Nutrition,
233 130, 396S-402S.

234 Sandi, S., Yosi, F., Sari, M.L. and Gofar, N., 2018. The characteristics and potential of lactic acid bacteria as
235 probiotics in silage made from *Hymenachne acutigluma* and *Neptunia oleracea* lour. In:, E3S Web of
236 Conferences.

237 Sandi, S., Yosi, F., Sari, M.L., Gofar, N. and Rofiq, M.N., 2020. Swamp forage silage organic acid profiles and

238 influence of silage liquid organic acid salts against pathogenic bacteria in vitro. International Journal of
239 Poultry Science, 19, 147–152

240 Soltan, M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood
241 serum parameters in laying hens. International Journal of Poultry Sciences, 7, 613–621.

242 Sudaryani, T., 2000. Kualitas telur, (Penebar Swadaya: Jakarta).

243 Sumiati, S., Darmawan, A. and Hermana, W., 2020. Performances and egg quality of laying ducks fed diets
244 containing cassava (*Manihot esculenta* Crantz) leaf meal and golden snail (*Pomacea canaliculata*). Tropical
245 Animal Science Journal, 43, 227–232 .

246 Zahri, I. and Febriansyah, A., 2014. Business diversification and its impact on household income of farmers in
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248 Ziaie, H., Bashtani, M., Torshizi, M.A.K., Naeemipour, H., Farhangfar, H., Zeinali, A. and others, 2011. Effect
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251

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	<i>P</i> 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).

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 supplementation 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;

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Commented [A2]: 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.

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

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

46 Lowland farming serves potential contributions for the sustainability of food production in South
47 Sumatra. The agroecological zone is characterized mainly by the high acidity of land and water and periodic
48 flooding during the rainy season. The Pegagan duck is considered more suitable for small-scale farmers in the
49 region. The waterfowl provide egg, meat, and additional daily income for the farmers (Zahri and Febriansyah,
50 2014). However, duck eggs contain higher fat and cholesterol than chicken eggs. With increasing living standards
51 in recent years, people focus more on the healthy and high quality of eggs. The high concentration of cholesterol
52 in the duck egg is the main consideration for old people and hypercholesterolemia patients. An excess intake of
53 cholesterol may result in the disease of atherosclerosis and fatty liver (Danahy, 2020).

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

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

62

63 **Materials and Methods**

64 **Study site**

65 An animal feeding experiment was conducted for 10 weeks at the experimental station, Department of
66 Animal Science, Faculty of Agriculture, Universitas Sriwijaya. The ducks were cared for according to the Animal
67 Welfare Guidelines of the Indonesian Institute of Sciences. The approval of the experiment was granted from
68 Universitas Sriwijaya.

69

70 **Experimental animal and treatments**

71 Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks were weighed
72 and then allotted randomly into six treatments, with four replicates per treatment and three ducks in each replicate.

73 The ducks were kept in experimental pens (1.5 m², ~~four~~three birds in each pen) with 5 cm of rice husk litter. The
74 pens were located in an open-sided barn after proper cleaning and disinfection. The ~~animals~~birds were fed with
75 a basal diet (NRC, 1994) that was processed as crumble. The composition and nutrient concentrations of the basal
76 diet are shown in Table 1. The treatments were: basal diet, basal diet + organic acid, basal diet + probiotic, basal
77 diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic acid + tetracycline. Feed and
78 water were provided ad libitum while the artificial lighting was provided from 18:00 to 6:00 h.24 h.

79 Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2
80 %, v/v) was added in drinking water. The probiotic preparation was referred to the method of Bregni et al. (2000).

81 The silage was prepared from a local swamp grass (Kumpai Tembaga grass, *Hymenachne acutigluma*) plus 3%
82 molasses (w/w, fresh matter basis) (Sandi et al., 2018). An isolate from the silage was incubated in the deMann
83 Rogosa Sharp medium for 48 hours. The culture was centrifuged at 3000 rpm for 15 minutes to separate the
84 supernatant and substrate. The substrate contained ~~had~~ 8.24 colony-forming unit/ml of lactic acid bacteria per ml.

85 An amount of 5% (w/w) of the mixture of milk skim and maltodextrin was added to the substrate. Afterward, the
86 substrate was dried at 37 °C for 48 hours. Organic acid preparation was referred to Negara (2009): The distilled
87 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
88 refrigerator (5 °C) before use. Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of
89 acetic acid (v/v).

Commented [A4]: 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.

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Commented [A6]: Composition of organic acid and probiotic needs to be mentioned.

90

91 **Sampling and handling**

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

97 **Laboratory analyses and measurement methods**

98 Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract ether (AOAC,
99 2005). Calcium, phosphorus, and potassium according to the methods of AOAC (2005). Calcium and potassium
100 concentrations of Ca and Mg were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800
101 while P-phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer Lambda 45 (AOACe,
102 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance
103 (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were measured according
104 to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg
105 quality parameters were measured in all cases within 48 hours of collection.

106 **Statistical analysis**

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

111

112 **Result and discussion**

113 The organic acid and probiotic supplementation had no effects on feed intake, laying rate, and feed-to-
114 egg ratio (Data not shown). Egg weight, albumen weight, and yolk weight were also not different ($P > 0.05$)
115 among the treatments while eggshell weight was higher ($P < 0.05$) in the probiotic and organic acid+probiotic
116 treatments compared to the basal diet treatment (Table 2). The range of the eggshell weight in the present study
117 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
118 than the range reported by Etuk et al. (2012) in Muscovy duck (7.01 to 7.06 g). The highest eggshell weight was
119 obtained in the organic acid + probiotic supplementation. Though the present study did not measure calcium and

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

125 The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score ranged from
126 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
127 work could be categorized as good quality egg according to Sudaryani (2000) where the good color score range
128 from 9.00 to 12.00. The highest mean of the colour index was found in the organic acid+probiotic treatment. The
129 improvement of nutrient absorption due to the combination of the organic and probiotic supplementation might
130 related to a higher absorption of beta carotene. The yolk index of the duck in this study ranged from 0.38 to 0.41.
131 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
132 mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that
133 regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index value was in line with the
134 protein contents of the egg in the present study where the highest protein content was also found in the organic
135 acid+probiotic treatment while the lowest in the basal diet.

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

141 The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and
142 cholesterol content ~~in the yolk~~ ($P < 0.01$). The range of Haugh unit score in the present work was similar to the
143 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
144 Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study.
145 The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic
146 acid+probiotic supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic
147 acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a
148 result of the biological and chemical activity produced by the organic acid+probiotic supplementation, where the
149 accumulation of these two substrates causes changes in the lipid profile in the digestive systemtract. According to

150 previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could assimilate the cholesterol in the
151 intestines for cell metabolism and thus reducing the total absorption of lipid. Moreover, Ooi and Liang, (2010)
152 reviewed that A study conducted to determine the effect of using probiotics on lowering cholesterol levels reported
153 that There are 3 three mechanisms that could cause the decrease in cholesterol and fat levels: 1), namely 1),
154 inhibition of cholesterol synthesis due to probiotic fermented compounds; 2) Bbile salt deconjugation, which
155 causes a higher use of cholesterol for bile salt synthesis, and and results in lower cholesterol levels; 3) t. The
156 existence of the ability of probiotics to bind cholesterol, causing low cholesterol levels. Furthermore, The
157 decreased fat and cholesterol content were consistent with the previous study using layer chickens (Mohan et al.,
158 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study by Li et al. (2011) was also reported that supplementation
159 of *Bacillus subtilis* in the diet of Shaoxing duck reduced the yolk cholesterol content of eggs from 126.96 to 97.09
160 mmol/L. The role of probiotics in reducing egg cholesterol could be related to the changing lipid metabolism in
161 the digestive tract. According to previous studies (Catherine et al., 2014; Park et al., 2018), probiotics could
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163 Finally, this study shows that organic acid and probiotic supplementations affected yolk color, yolk fat,
164 and cholesterol contents. The yolk color was improved by organic acid and probiotic supplementations where the
165 highest yolk color index was found in organic acid+probiotic supplementation. The yolk fat and cholesterol
166 content were lower than those in the other supplementations.

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

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174 **Data availability** on request.

175 **Code availability** not applicable

176 **Declarations**

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

179 **Consent to participate** not applicable

180 **Consent for publication** not applicable

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

182 **References**

183 Abbas, G., Khan, S.H. and Rehman, H., 2013. Effects of formic acid administration in the drinking water on
184 production performance, egg quality and immune system in layers during hot season. *Avian Biology*
185 *Research*, 6, 227–232.

186 Abdelqader, A., Irshaid, R. and Al-Fataftah, A.-R., 2013. Effects of dietary probiotic inclusion on performance,
187 eggshell quality, cecal microflora composition, and tibia traits of laying hens in the late phase of production.
188 *Tropical Animal Health and Production*, 45, 1017–1024.

189 Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K., 1996. The influence of
190 *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of
191 plasma and egg yolk. *British Poultry Science*, 37, 341–346.

192 [Adil, S., Banday, T., Bhat, G.A., Mir, M.S. and Rehman, M., 2010. Effect of dietary supplementation of organic
193 acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary
194 Medicine International*, 2010, 479485](#)

195 AOAC, 2005. Official Methods of Analysis of AOAC International. Official Methods of Analysis, (AOAC:
196 Gaithersburg, MD).

197 Bell D. 2002. Formation of the egg. In: Bell DD, Weaver WD, editors. Commercial chicken meat and egg
198 production 5th edition volume II, (Springer Science: New York).

199 Bregni, C., Degrossi, J., Garcia, R., Lamas, M.C., Firenstein, R., D'aquino, M. and others, 2000. Alginate
200 microspheres of *Bacillus subtilis*. *Ars Pharmaceutica*, 41, 245–248

201 Card, L.E. and Nesheim, M., 1972. *Poultry Production*, (Lea and febiger Press: Phi delphia).

202 Catherine, T.D., Jones, M.L., Shah, D., Jain, P., Saha, S. and Prakash, S., 2014. Cholesterol assimilation by
203 *Lactobacillus* probiotic bacteria: An in vitro investigation. *BioMed Research International*, 2014, 1–9.

204 Danahy, A., 2020. Duck eggs: Nutrition, benefits, and side effects. <https://www.healthline.com/nutrition/duck-eggs#nutrition>. Accessed 19 April 2020.

206 Dibner, J.J. and Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition
207 and metabolism. *Journal of Applied Poultry Research*, 11, 453–463.

208 Diplock, A.T., Symons, M.C.R. and Rice-Evans, C.A., 1991. *Techniques in free radical research*, (Elsevier
209 Science).

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210 Etuk, I.F., Ojewola, G.S., Abasiokong, S.F., Amaefule, K.U. and Etuk, E.B., 2012. Egg quality of Muscovy ducks
 211 reared under different management systems in the humid tropics. *Revista Científica UDO Agrícola*, 12,
 212 226–229.

213 Fouad, A.M., Ruan, D., Lin, Y.C., Zheng, C.T., Zhang, H.X., Chen, W., Wang, S., Xia, W.G. and Li, Y., 2016.
 214 Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying ducks.
 215 *British Poultry Science*, 57, 818–823.

216 Fuller, M.F. and Cadenhead, A., 1991. Effect of the amount and composition of the diet on galactosamine flow
 217 from the small intestine. (EAAP: April 24 - 26. Wageningen, Netherlands. p 330).

218 Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S., 1997. Probiotics in poultry: modes of action. *World's Poultry
 219 Science Journal*, 53, 351–368.

220 Li, W.F., Rajput, I.R., Xu, X., Li, Y.L., Lei, J., Huang, Q. and Wang, M.Q., 2011. Effects of probiotic (*Bacillus
 221 subtilis*) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck.
 222 *International Journal of Poultry Science*, 10, 583–589.

223 Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A., 1995. Effect of probiotic supplementation on
 224 serum/yolk cholesterol and on egg shell thickness in layers. *British Poultry Science*, 36, 799–803.

225 Negara, W. 2009. Study of Organic Acid Salt Production from Complete Ration Silage as a Growth Booster in
 226 Broilers. M.Sc Thesis. IPB. Bogor. Indonesia.

227 NRC, 1994. Nutrient requirements of poultry. National Research Council, (National Academy Press: Washington
 228 D.C)

229 [Ooi, L.-G. and Liong, M.-T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo
 230 and in vitro findings. *International Journal of Molecular Sciences*, 11, 2499–2522](#)

231 Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y.G., Kim, A.R., Holzappel, W. and Ji, Y., 2018.
 232 Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome
 233 and propionate level in a murine model. *PLoS ONE*, 13.

234 Patel, S.G., Raval, A.P., Bhagwat, S.R., Sadrasaniya, D.A., Patel, A.P. and Joshi, S.S., 2015. Effects of probiotics
 235 supplementation on growth performance, feed conversion ratio and economics of broilers. *Journal of
 236 Animal Research*, 5, 155.

237 Pelicano, E., Souza, P. de, Souza, H. de, Oba, A., Norkus, E., Kodawara, L. and Lima, T. de, 2003. Effect of
 238 different probiotics on broiler carcass and meat quality. *Revista Brasileira de Ciência Avícola*, 5, 207–214.

239 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical

240 Computing. (Vienna, Austria).

241 Ricke, S., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry
242 Science, 82, 632–639.

243 Rolfe, R.D., 2000. The role of probiotic cultures in the control of gastrointestinal health. The Journal of Nutrition,
244 130, 396S-402S.

245 Sandi, S., Yosi, F., Sari, M.L. and Gofar, N., 2018. The characteristics and potential of lactic acid bacteria as
246 probiotics in silage made from *Hymenachne acutigluma* and *Neptunia oleracea* Lour. In., E3S Web of
247 Conferences.

248 [Sandi, S., Yosi, F., Sari, M.L., Gofar, N. and Rofiq, M.N., 2020. Swamp forage silage organic acid profiles and
249 influence of silage liquid organic acid salts against pathogenic bacteria in vitro. International Journal of
250 Poultry Science, 19, 147–152](#)

251 Soltan, M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood
252 serum parameters in laying hens. International Journal of Poultry Sciences, 7, 613–621.

253 Sudaryani, T., 2000. Kualitas telur, (Penebar Swadaya: Jakarta).

254 Sumiati, S., Darmawan, A. and Hermana, W., 2020. Performances and egg quality of laying ducks fed diets
255 containing cassava (*Manihot esculenta* Crantz) leaf meal and golden snail (*Pomacea canaliculata*). Tropical
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	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>

Decision on your manuscript #TROP-D-21-00659R1

André Martinho de Almeida <em@editorialmanager.com>

10 September 2021 pukul 18.32

Balas Ke: André Martinho de Almeida <andrealmeidaredirect@gmail.com>

Kepada: asep indra munawar ali <asep_ali@fp.unsri.ac.id>

Dear Dr. ali:

We have received the reports from our advisors on your manuscript, "Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck", which you submitted to Tropical Animal Health and Production.

Based on the advice received, your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions.

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Tropical Animal Health and Production

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Tropical Animal Health and Production <em@editorialmanager.com>

2 Oktober 2021 pukul 12.24

Balas Ke: Tropical Animal Health and Production <mariafe.delaserna@springernature.com>

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Decision on your Manuscript #TROP-D-21-00659R2

André Martinho de Almeida <em@editorialmanager.com>

4 Desember 2021 pukul 01.01

Balas Ke: André Martinho de Almeida <andrealmeidaredirect@gmail.com>

Kepada: asep indra munawar ali <asep_ali@fp.unsri.ac.id>

RE:

#TROP-D-21-00659R2

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

Dear Dr. ali:

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Yours sincerely,

Sezen Özkan, Ph.D.

Tropical Animal Health and Production

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Tropical Animal Health and Production <em@editorialmanager.com>

29 Desember 2021 pukul 15.26

Balas Ke: Tropical Animal Health and Production <mariafe.delaserna@springernature.com>

Kepada: asep indra munawar ali <asep_ali@fp.unsri.ac.id>

Dear Dr. ali:

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Organic acid and probiotic derived from grass silage improved egg quality in Pegagan laying duck: a research note", submitted to Tropical Animal Health and Production. The manuscript number is TROP-D-21-00659R3.

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Tropical Animal Health and Production

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

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	
Corresponding Author:	asep indra munawar ali, Ph.D. Universitas Sriwijaya Fakultas Pertanian Ogan Ilir, None Selected INDONESIA	
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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 & 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 substrate with 8.24 CFU.	82	Yes, selective medium. The spesies of lactic acid bacteria (<i>Lactobacillus plantarum</i>) 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	

31 performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful
32 microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal by
33 stimulating the synthesis of vitamins B-group, improving immunity, and increasing volatile fatty acids.

34 Pancreatic secretion was increased by organic acids supplementation (Dibner and Buttin, 2002). Villus
35 height in the small intestines and serum calcium and phosphorus concentrations were also elevated (Adil et al.,
36 2010). Organic acids supplementation was associated with lowering the pH of the digestive tract and related to
37 reduction of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH
38 was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke,
39 2003). Moreover, organic acids supplementation improved feed efficiency, egg mass, eggshell quality, and yolk
40 index in layer hen (Soltan, 2008).

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

49 To our knowledge, the effects of supplementation of probiotics, organic acids, and their combination to
50 the duck egg quality parameters have not been tested. Organic acids and lactic acid bacteria derived from grass
51 silage might serve as a valuable alternative of additive since simple and less expensive preparation (Sandi et al.,
52 2020). The organic acids could serve not only for lowering pH when the digestive tracts were already acidic.
53 Therefore, the objectives of this study were to investigate the effects of supplementation of probiotics and
54 organic acids derived from grass silage on egg quality parameters of Pegagan duck and compare the
55 effectiveness of supplements and antibiotic tetracycline.

56

57 **Materials and Methods**

58 **Experimental animal and treatments**

59 An experimental trial was conducted for 10 weeks at the experimental station, Faculty of Agriculture,
60 Universitas Sriwijaya. Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks

61 were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks
62 in each replicate. The ducks were kept in experimental pens (1.5 m², three birds in each pen) in an open-sided
63 barn with 5 cm of rice husk litter after proper cleaning and disinfection. The birds were fed with a basal diet
64 (NRC, 1994) that was processed as crumble (Table 1). The treatments were: basal diet, basal diet + organic acid,
65 basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet + organic
66 acid + tetracycline. Feed and water were provided ad libitum while the artificial lighting was provided from
67 18:00 to 6:00 h.

68 Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2
69 %, v/v) was added in drinking water. The silage was prepared from a swamp grass (*Hymenachne acutigluma*)
70 plus 3% molasses (w/w, fresh matter basis). An isolate from the silage was incubated in the deMann Rogosa
71 Sharp medium (a selective medium for lactic acid bacteria) for 48 hours. The culture was centrifuged at 3000
72 rpm for 15 minutes to separate the supernatant and substrate. The substrate contained 8.24 colony-forming unit
73 of *Lactobacillus plantarum* per ml. The substrate was mixed with an amount of 5% (w/w) of the mixture of milk
74 skim and maltodextrin and then dried at 37 °C for 48 hours (Sandi et al., 2018). Organic acid was prepared by
75 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
76 the refrigerator (5 °C). Total acid concentration was 3.8% that consisted of 2.78% of lactic acid and 1% of acetic
77 acid (v/v) (Sandi et al., 2020).

78 **Laboratory analyses and measurement methods**

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

83 Samples of basal diet were analyzed for dry matter, crude protein, crude fiber, and extract. Calcium and
84 potassium concentrations were analyzed using an atomic absorption spectrophotometer Shimadzu AA-6800
85 while phosphorus concentration was analyzed used a spectrophotometer Perkin Elmer Lambda 45 (AOAC,
86 2005). Egg weight, albumen weight, yolk weight, and shell weight were measured with an electronic balance
87 (Ohaus CP214). Yolk colour score, egg index, yolk index, albumen index, Haugh unit were measured according
88 to Card and Nesheim (1972) whilst cholesterol content was analyzed according to Diplock et al. (1991). Egg
89 quality parameters were measured in all cases within 48 hours of collection.

90 **Statistical analysis**

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

94

95 **Result and discussion**

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

108 The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score was higher
109 than that in the egg of the Pajajaran duck (5.56 to 8.89) and could be categorized as good quality egg (range
110 from 9.00 to 12.00) (Sudaryani, 2000). The highest mean of the colour index was found in the organic
111 acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and
112 probiotic supplementation might related to a higher absorption of beta carotene. The yolk index of the duck in
113 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
114 mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that
115 regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index values were in line with
116 the protein contents of the egg where the highest protein content was also found in the organic acid+probiotic
117 treatment while the lowest in the basal diet.

118 The organic acid supplementation improved Haugh unit and the cholesterol content in the yolk ($P <$
119 0.05) though the drinking water was already acidic. The lactic and acetic acid in the acid supplementation could

120 improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the
121 previous studies reported (Mohan et al., 1995; Abdulrahim et al., 1996).

122 The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and
123 cholesterol content ($P < 0.01$). The range of Haugh unit score in the present work was similar to the range in the
124 study of Sumiati et al. (2020) (90.00 to 93.53) but higher than a study of Fouad et al. (2016) in Longyan ducks
125 (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study. The highest
126 Haugh unit score was in line with the highest value of the egg weight and albumen weight in organic
127 acid+probiotic supplementation (Table 2). The decrease fat and cholesterol in the yolk with organic
128 acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents (Adil et al., 2010) as a
129 result of biological and chemical activity produced by the organic acid+probiotic in the digestive tract.
130 According to previous studies (Tomaro-Duchesneau et al., 2014; Park et al., 2018), probiotics could assimilate
131 the cholesterol in the intestines for cell metabolism and thus reducing the total absorption of lipid. Moreover,
132 Ooi and Liong (2010) reviewed that three mechanisms could cause the decrease in cholesterol and fat levels: 1)
133 inhibition of cholesterol synthesis due to probiotic fermented compounds, 2) bile salt deconjugation, which
134 causes a higher use of cholesterol for bile salt synthesis, and 3) the ability of probiotics to bind cholesterol.
135 Furthermore, the decreased fat and cholesterol content were consistent with the previous study using layer
136 chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study in Shaoxing duck also reported
137 that supplementation of *Bacillus subtilis* reduced the yolk cholesterol content of eggs from 126.96 to 97.09
138 mmol/L by (Li et al., 2011).

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

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

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

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

149 **Data availability** on request.

150 **Code availability** not applicable

151 **Declarations**

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

154 **Consent to participate** not applicable

155 **Consent for publication** not applicable

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

157 **References**

158 Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K., 1996. The influence of
159 *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of
160 plasma and egg yolk. *British Poultry Science*, 37, 341–346

161 Adil, S., Banday, T., Bhat, G.A., Mir, M.S. and Rehman, M., 2010. Effect of dietary supplementation of organic
162 acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary
163 Medicine International*, 2010, 479485 (SAGE-Hindawi Access to Research)

164 AOAC, 2005. *Official Methods of Analysis of AOAC International*. Official Methods of Analysis, (AOAC:
165 Gaithersburg, MD).

166 Bell, D.D., 2002. Formation of the egg. In: D. D. Bell and W. . Weaver (eds), *Commercial Chicken Meat and
167 Egg Production*. (Springer Science: New York)

168 Card, L.E. and Nesheim, M., 1972. *Poultry Production*, (Lea and febiger Press: Philadelphia)

169 Danahy, A., 2020. Duck eggs: Nutrition, benefits, and side effects. [https://www.healthline.com/nutrition/duck-](https://www.healthline.com/nutrition/duck-eggs#nutrition)
170 [eggs#nutrition](https://www.healthline.com/nutrition/duck-eggs#nutrition). Accessed 19 April 2020.

171 Dibner, J.J. and Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on
172 nutrition and metabolism. *Journal of Applied Poultry Research*, 11, 453–463

173 Diplock, A.T., Symons, M.C.R. and Rice-Evans, C.A., 1991. *Techniques in free radical research*, (Elshevier)

174 Etuk, I.F., Ojewola, G.S., Abasiokong, S.F., Amaefule, K.U. and Etuk, E.B., 2012. Egg quality of Muscovy
175 ducks reared under different management systems in the humid tropics. *Revista Científica UDO Agrícola*,
176 12, 226–229

177 Fouad, A.M., Ruan, D., Lin, Y.C., Zheng, C.T., Zhang, H.X., Chen, W., Wang, S., Xia, W.G. and Li, Y., 2016.
178 Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying
179 ducks. *British Poultry Science*, 57, 818–823

180 Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S., 1997. Probiotics in poultry: modes of action. World's
181 Poultry Science Journal, 53, 351–368

182 Li, W.F., Rajput, I.R., Xu, X., Li, Y.L., Lei, J., Huang, Q. and Wang, M.Q., 2011. Effects of probiotic (*Bacillus*
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184 International Journal of Poultry Science, 10, 583–589

185 Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A., 1995. Effect of probiotic supplementation on
186 serum/yolk cholesterol and on egg shell thickness in layers. British Poultry Science, 36, 799–803

187 NRC, 1994. Nutrient requirements of poultry. National Research Council, (National Academy Press:
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189 Ooi, L.-G. and Liong, M.-T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: A review of in
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191 Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y.G., Kim, A.R., Holzapfel, W. and Ji, Y., 2018.
192 Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome
193 and propionate level in a murine model.

194 Patel, S.G., Raval, A.P., Bhagwat, S.R., Sadrasaniya, D.A., Patel, A.P. and Joshi, S.S., 2015. Effects of
195 probiotics supplementation on growth performance, feed conversion ratio and economics of broilers.
196 Journal of Animal Research, 5, 155

197 R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical
198 Computing. (Vienna, Austria)

199 Ricke, S., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry
200 Science, 82, 632–639

201 Sandi, S., Yosi, F., Sari, M.L. and Gofar, N., 2018. The characteristics and potential of lactic acid bacteria as
202 probiotics in silage made from *Hymenachne acutigluma* and *Neptunia oleracea* Lour. In: E3S Web of
203 Conferences.

204 Sandi, S., Yosi, F., Sari, M.L., Gofar, N. and Rofiq, M.N., 2020. Swamp forage silage organic acid profiles and
205 influence of silage liquid organic acid salts against pathogenic bacteria in vitro. International Journal of
206 Poultry Science, 19, 147–152

207 Soltan, M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some
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209 Sudaryani, T., 2000. Kualitas telur, (Penebar Swadaya: Jakarta)

- 210 Sumiati, S., Darmawan, A. and Hermana, W., 2020. Performances and egg quality of laying ducks fed diets
211 containing cassava (*Manihot esculenta* Crantz) leaf meal and golden snail (*Pomacea canaliculata*).
212 Tropical Animal Science Journal, 43, 227–232
- 213 Tomaro-Duchesneau, C., Jones, M.L., Shah, D., Jain, P., Saha, S. and Prakash, S., 2014. Cholesterol
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215 2014, 1–9
- 216 Zahri, I. and Febriansyah, A., 2014. Business diversification and its impact on household income of farmers in
217 swampy lowland areas. Agricultural Socio-Economics Journal, 14, 144–153
- 218 Ziaie, H., Bashtani, M., Torshizi, M.A.K., Naeemipour, H., Farhangfar, H., Zeinali, A. and others, 2011. Effect
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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

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

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225

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

Parameters	Experimental treatments						SEM	<i>P</i> 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).

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

3
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13

14 **Abstract**

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

25 **Keyword** Probiotics, Organic Acid Salt, Egg Quality, Pegagan Duck
26

27 **Introduction**

28 Probiotic supplementation has been found to improve growth performance and feed conversion ratio of
29 broilers (Patel et al., 2015), egg size, egg mass, egg weight (Jin et al., 1997) and ~~and~~ reduce egg yolk cholesterol
30 in laying hen (Mohan et al., 1995; Abdulrahim et al., 1996). The utilization of probiotics could improve the

31 performance of digestive enzymes and maintain intestinal microflora by competitive exclusion to harmful
32 microbes, altering metabolism, and decreasing ammonia production. Probiotics benefit the host animal host by
33 stimulating the synthesis of vitamins B-group, improving host-immunity, and increasing volatile fatty acids.

34 Pancreatic secretion was increased by organic acids supplementation (Dibner and Buttin, 2002). Villus
35 height in the small intestines and serum calcium and phosphorus concentrations were also elevated (Adil et al.,
36 2010). Organic acids supplementation was associated with lowering the pH of the digestive tract and related to
37 reductions of acid-intolerant bacteria such as *Escherichia coli*, *Salmonella* and *Campylobacter*. The lower pH
38 was also related to an increase of amino acid absorptions in the small intestine (Dibner and Buttin, 2002; Ricke,
39 2003). Moreover, organic acids supplementation improved feed efficiency, egg mass, eggshell quality, and yolk
40 index in layer hen (Soltan, 2008).

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

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

57

58 **Materials and Methods**

59 **Experimental animal and treatments**

60 An experimental trial was conducted for 10 weeks at the experimental station, Faculty of Agriculture,
61 Universitas Sriwijaya. Laying Pegagan ducks (n: 72, 24 weeks old) were provided by a local breeder. The ducks
62 were weighed and then allotted randomly into six treatments, with four replicates per treatment and three ducks
63 in each replicate. The ducks were kept in experimental pens (1.5 m², ~~four-three~~ birds in each pen) in an open-
64 sided barn with 5 cm of rice husk litter after proper cleaning and disinfection. The ~~animals-birds~~ were fed with a
65 basal diet (NRC, 1994) that was processed as crumble (Table 1). The treatments were: basal diet, basal diet +
66 organic acid, basal diet + probiotic, basal diet + tetracycline, basal diet + organic acid + probiotic, and basal diet
67 + organic acid + tetracycline. Feed and water were provided ad libitum while the ~~artificial~~ lighting was provided
68 ~~from 18:00 to 6:00 h.24 h.~~

69 Probiotic and tetracycline (0.2 %, dry matter basis) were mixed in the basal diet while organic acid (0.2
70 %, v/v) was added in drinking water. The silage was prepared from a swamp grass (*Hymenachne acutigluma*)
71 plus 3% molasses (w/w, fresh matter basis). An isolate from the silage was incubated in the deMann Rogosa
72 Sharp medium (a selective medium for lactic acid bacteria) for 48 hours. The culture was centrifuged at 3000
73 rpm for 15 minutes to separate the supernatant and substrate. ~~The substrate contained had-8.24 colony-forming~~
74 ~~unit/ml of *Lactobacillus plantarum* per ml.~~ The substrate was mixed with an amount of 5% (w/w) of the mixture
75 of milk skim and maltodextrin and then dried at 37 °C for 48 hours (Sandi et al., 2018). Organic acid was
76 prepared by mixing the distilled water to the silage (1:1, w/w). The mixture (pH: 3.8 to 5.0) was then filtered
77 and stored in the refrigerator (5 °C). ~~Total acid concentration was 3.8% that consisted of 2.78% of lactic acid~~
78 ~~and 1% of acetic acid (v/v)~~ (Sandi et al., 2020).

79 **Laboratory analyses and measurement methods**

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

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

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

92 **Statistical analysis**

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

96

97 **Result and discussion**

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

110 The yolk colour was affected ($P < 0.01$) by the treatments (Table 2). The yolk colour score was higher
111 than that in the egg of the Pajajaran duck (5.56 to 8.89) and could be categorized as good quality egg (range
112 from 9.00 to 12.00) (Sudaryani, 2000). The highest mean of the colour index was found in the organic
113 acid+probiotic treatment. The improvement of nutrient absorption due to the combination of the organic and
114 probiotic supplementation might related to a higher absorption of beta carotene. The yolk index of the duck in
115 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
116 mainly influenced by the protein content of diet which can stimulate the formation of membrane vitellin that
117 regulates the transfer of water between yolk and albumen (Bell, 2002). The yolk index values were in line with

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118 the protein contents of the egg where the highest protein content was also found in the organic acid+probiotic
119 treatment while the lowest in the basal diet.

120 ~~The organic acid supplementation improved Haugh unit and the cholesterol content in the yolk ($P <$~~
121 ~~0.05) though the . It seems that the acid-drinking water might have less effect to the pH of the digestive tract but~~
122 ~~this needs further investigation~~ was already acidic. The lactic and acetic acid in the acid supplementation could
123 ~~improve amino acid absorption or affected the metabolism of fat and cholesterol in the digestive tracts as the~~
124 ~~previous studies reported~~ (Mohan et al., 1995; Abdulrahim et al., 1996).

125 The organic acid+probiotic supplementation improved the Haugh unit of the egg and decrease fat and
126 cholesterol content ~~in the yolk~~ ($P < 0.01$). The range of Haugh unit score in the present work was similar to the
127 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
128 Longyan ducks (69.40 to 72.20). The higher Haugh unit indicated a thicker albumen layer in the present study.
129 The highest Haugh unit score was in line with the highest value of the egg weight and albumen weight in
130 organic acid+probiotic supplementation (Table 2). ~~The decrease fat and cholesterol in the yolk with organic~~
131 ~~acid+probiotics presumably due to a reduction of serum and plasma cholesterol contents~~ (Adil et al., 2010) ~~as a~~
132 ~~result of the biological and chemical activity produced by the organic acid+probiotic supplementation, where~~
133 ~~the accumulation of these two substrates causes changes in the lipid profile in the digestive system tract.~~
134 ~~According to previous studies~~ (Tomaro-Duchesneau et al., 2014; Park et al., 2018), ~~probiotics could assimilate~~
135 ~~the cholesterol in the intestines for cell metabolism and thus reducing the total absorption of lipid. Moreover,~~
136 Ooi and Liong (2010) ~~reviewed that A study conducted to determine the effect of using probiotics on lowering~~
137 ~~cholesterol levels reported that tThere are 3 tThree mechanisms that could cause the decrease in cholesterol~~
138 ~~and fat levels: 1), namely 1): inhibition of cholesterol synthesis due to probiotic fermented compounds,; 2) :~~
139 ~~Bbile salt deconjugation, which causes a higher use of cholesterol for bile salt synthesis, and -and results in~~
140 ~~lower cholesterol levels; 3) t: The existence of the ability of probiotics to bind cholesterol, causing low~~
141 ~~cholesterol levels-. Furthermore, tThe decreased fat and cholesterol content were consistent with the previous~~
142 study using layer chickens (Mohan et al., 1995; Abdulrahim et al., 1996; Jin et al., 1997). A study in Shaoxing
143 duck also reported that supplementation of *Bacillus subtilis* reduced the yolk cholesterol content of eggs from
144 126.96 to 97.09 mmol/L by (Li et al., 2011) ~~was. The role of probiotics in reducing egg cholesterol could be~~
145 ~~related to the changing lipid metabolism in the digestive tract. According to previous studies (Catherine et al.,~~
146 ~~2014; Park et al., 2018), probiotics could assimilate the cholesterol in the intestines for cell metabolism and~~
147 ~~thus reducing the total absorption of lipid.~~

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

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

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158 **Data availability** on request.

159 **Code availability** not applicable

160 **Declarations**

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

163 **Consent to participate** not applicable

164 **Consent for publication** not applicable

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

166 **References**

167 Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K., 1996. The influence of
168 *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of
169 plasma and egg yolk. *British Poultry Science*, 37, 341–346

170 Adil, S., Banday, T., Bhat, G.A., Mir, M.S. and Rehman, M., 2010. Effect of dietary supplementation of organic
171 acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary
172 Medicine International*, 2010, 479485 (SAGE-Hindawi Access to Research)

173 AOAC, 2005. *Official Methods of Analysis of AOAC International*. Official Methods of Analysis, (AOAC:
174 Gaithersburg, MD).

175 Bell, D.D., 2002. Formation of the egg. In: D. D. Bell and W. . Weaver (eds), *Commercial Chicken Meat and
176 Egg Production*. (Springer Science: New York)

177 Card, L.E. and Nesheim, M., 1972. *Poultry Production*, (Lea and febiger Press: Philadelphia)

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178 Danahy, A., 2020. Duck eggs: Nutrition, benefits, and side effects. [https://www.healthline.com/nutrition/duck-](https://www.healthline.com/nutrition/duck-eggs#nutrition)
179 [eggs#nutrition](https://www.healthline.com/nutrition/duck-eggs#nutrition). Accessed 19 April 2020.

180 Dibner, J.J. and Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on
181 nutrition and metabolism. *Journal of Applied Poultry Research*, 11, 453–463

182 Diplock, A.T., Symons, M.C.R. and Rice-Evans, C.A., 1991. *Techniques in free radical research*, (Elsevier)

183 Etuk, I.F., Ojewola, G.S., Abasiokong, S.F., Amaefule, K.U. and Etuk, E.B., 2012. Egg quality of Muscovy
184 ducks reared under different management systems in the humid tropics. *Revista Científica UDO Agrícola*,
185 12, 226–229

186 Fouad, A.M., Ruan, D., Lin, Y.C., Zheng, C.T., Zhang, H.X., Chen, W., Wang, S., Xia, W.G. and Li, Y., 2016.
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189 Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S., 1997. Probiotics in poultry: modes of action. *World's*
190 *Poultry Science Journal*, 53, 351–368

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192 *subtilis*) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck.
193 *International Journal of Poultry Science*, 10, 583–589

194 Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A., 1995. Effect of probiotic supplementation on
195 serum/yolk cholesterol and on egg shell thickness in layers. *British Poultry Science*, 36, 799–803

196 NRC, 1994. *Nutrient requirements of poultry*. National Research Council, (National Academy Press:
197 Washington D.C)

198 Ooi, L.-G. and Liong, M.-T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: A review of in
199 vivo and in vitro findings. *International Journal of Molecular Sciences*, 11, 2499–2522

200 Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y.G., Kim, A.R., Holzappel, W. and Ji, Y., 2018.
201 Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome
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203 Patel, S.G., Raval, A.P., Bhagwat, S.R., Sadrasaniya, D.A., Patel, A.P. and Joshi, S.S., 2015. Effects of
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205 *Journal of Animal Research*, 5, 155

206 R Core Team, 2018. *R: A language and environment for statistical computing*. R Foundation for Statistical
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208 Ricke, S., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry
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211 probiotics in silage made from *Hymenachne acutigluma* and *Neptunia oleracea* Lour. In: E3S Web of
212 Conferences.

213 Sandi, S., Yosi, F., Sari, M.L., Gofar, N. and Rofiq, M.N., 2020. Swamp forage silage organic acid profiles and
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218 Sudaryani, T., 2000. Kualitas telur, (Penebar Swadaya: Jakarta)

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223 assimilation by Lactobacillus probiotic bacteria: An in vitro investigation. BioMed Research International,
224 2014, 1–9

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227 Ziaie, H., Bashtani, M., Torshizi, M.A.K., Naeemipour, H., Farhangfar, H., Zeinali, A. and others, 2011. Effect
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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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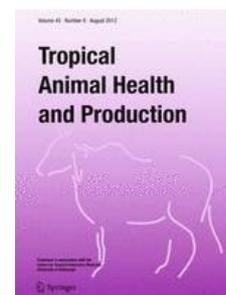
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Abstract The aim of this study was to evaluate the effect of supplementation of organic acid and probiotic derived from grass silage on the egg quality of duck. Seventy-two Pegagan laying ducks (average age: 24 weeks) were randomly allocated to six 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



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

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8 **Abstract**

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10 the egg quality of duck. Seventy-two Pegagan laying ducks (average age: 24 weeks) were randomly allocated to six treatment
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12 acid, and basal diet + organic acid + tetracycline. The result showed that the feeding diets containing probiotics and organic
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16 derived from grass silage improved egg quality in terms of yolk color score, fat, and cholesterol content.

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

18 **Introduction**

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

30 Pancreatic secretion was increased by organic acid sup-
31 plementation (Dibner and Buttin 2002). Villus height in the

small intestines and serum calcium and phosphorus concen- 32
trations were also elevated (Adil et al. 2010). Organic acid 33
supplementation was associated with lowering the pH of 34
the digestive tract and related to reduction of acid-intolerant 35
bacteria such as *Escherichia coli*, *Salmonella*, and *Campy-* 36
lobacter. The lower pH was also related to an increase of 37
amino acid absorptions in the small intestine (Dibner and 38
Buttin 2002; Ricke, 2003). Moreover, organic acid supple- 39
mentation improved feed efficiency, egg mass, eggshell qual- 40
ity, and yolk index in layer hen (Soltan 2008). 41

42 Lowland serves potential contributions for the farming 42
sustainability in South Sumatra. The agroecological zone is 43
characterized mainly by the high acidity of land and water 44
and periodic flooding. The Pegagan duck is considered more 45
suitable for small-scale farmers that provide egg, meat, and 46
additional daily income for the farmers (Zahri and Febri- 47
ansyah 2014). However, duck eggs contain higher fat and 48
cholesterol than chicken eggs. With increasing living stand- 49
ards in recent years, people focus more on the healthy and 50
high quality of eggs. The high concentration of cholesterol 51
in the duck egg is the main consideration for old people and 52
hypercholesterolemia patients. An excess intake of choles- 53
terol may result in the disease of atherosclerosis and fatty 54
liver (Danahy 2020). 55

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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 m², 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).

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 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)

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)

137 range of the eggshell weight was similar to the Sumiati et al.
 138 (2020) in the egg of local Pajajaran duck (7.97 to 8.46 g)
 139 but higher than the range reported by Etuk et al. (2012) in
 140 Muscovy duck (7.01 to 7.06 g). The highest eggshell weight
 141 was obtained in the organic acid + probiotic supplementa-
 142 tion; though the present study did not measure calcium and
 143 phosphorus content in the eggshell, this could be related to
 144 the higher digestion and absorption of calcium and phos-
 145 phorus in the lower pH of the digestive tracts (Soltan 2008).
 146 Supplementation of the mixture of organic acid (propionic
 147 acid and sodium bentonite) in the broiler diet caused an
 148 increase in the digestibility and availability of calcium and
 149 phosphorus due to developing desirable microflora (*Lacto-*
 150 *bacillus* sp) that results in increasing the mineral retention
 151 and bone mineralization (Ziaie et al. 2011).

152 The yolk color was affected ($P < 0.01$) by the treatments
 153 (Table 2). The yolk color score was higher than that in the
 154 egg of the Pajajaran duck (5.56 to 8.89) and could be cat-
 155 egorized as a good quality egg (range from 9.00 to 12.00)
 156 (Sudaryani 2000). The highest mean of the color index was
 157 found in the organic acid + probiotic treatment. The improve-
 158 ment of nutrient absorption due to the combination of the
 159 organic and probiotic supplementation might be related to
 160 a higher absorption of beta carotene. The yolk index of the
 161 duck in this study was lower than the range in the egg of
 162 Muscovy duck (0.40 to 0.41) (Etuk et al. 2012). Yolk index
 163 is mainly influenced by the protein content of diet which can
 164 stimulate the formation of membrane vitellin that regulates
 165 the transfer of water between yolk and albumen (Bell 2002).

166 The yolk index values were in line with the protein contents
 167 of the egg where the highest protein content was also found
 168 in the organic acid + probiotic treatment while the lowest in
 169 the basal diet.

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

177 The organic acid + probiotic supplementation improved
 178 the Haugh unit of the egg and decreased fat and cholesterol
 179 content ($P < 0.01$). The range of Haugh unit score in the
 180 present work was similar to the range in the study of Sum-
 181 iati et al. (2020) (90.00 to 93.53) but higher than a study
 182 of Fouad et al. (2016) in Longyan ducks (69.40 to 72.20).
 183 The higher Haugh unit indicated a thicker albumen layer in
 184 the present study. The highest Haugh unit score was in line
 185 with the highest value of the egg weight and albumen weight
 186 in organic acid + probiotic supplementation (Table 2). The
 187 decrease in fat and cholesterol in the yolk with organic
 188 acid + probiotics is presumably due to a reduction of serum
 189 and plasma cholesterol contents (Adil et al. 2010) as a result
 190 of biological and chemical activity produced by the organic
 191 acid + probiotic in the digestive tract. According to previous
 192 studies (Tomaro-Duchesneau et al. 2014; Park et al. 2018),
 193 probiotics could assimilate the cholesterol in the intestines
 194 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.

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References

Abdulrahim, S.M., Haddadin, M.S.Y., Hashlamoun, E.A.R. and Robinson, R.K., 1996. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. *British Poultry Science*, 37, 341–346

Adil, S., Bandy, T., Bhat, G.A., Mir, M.S. and Rehman, M., 2010. Effect of dietary supplementation of organic acids on

performance, intestinal histomorphology, and serum biochemistry of broiler chicken. *Veterinary Medicine International*, 2010, 479485 (SAGE-Hindawi Access to Research) 240

AOAC, 2005. Official Methods of Analysis of AOAC International. Official Methods of Analysis, (AOAC: Gaithersburg, MD). 241

Bell, D.D., 2002. Formation of the egg. In: D. D. Bell and W. . Weaver (eds), *Commercial Chicken Meat and Egg Production*. (Springer Science: New York) 242

Card, L.E. and Nesheim, M., 1972. *Poultry Production*, (Lea and febiger Press: Philadelphia) 243

Danahy, A., 2020. Duck eggs: Nutrition, benefits, and side effects. <https://www.healthline.com/nutrition/duck-eggs#nutrition>. Accessed 19 April 2020. 244

Dibner, J.J. and Buttin, P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *Journal of Applied Poultry Research*, 11, 453–463 245

Diplock, A.T., Symons, M.C.R. and Rice-Evans, C.A., 1991. Techniques in free radical research, (Elshevier) 246

Etuk, I.F., Ojewola, G.S., Abasiokong, S.F., Amaefule, K.U. and Etuk, E.B., 2012. Egg quality of Muscovy ducks reared under different management systems in the humid tropics. *Revista Científica UDO Agrícola*, 12, 226–229 247

Fouad, A.M., Ruan, D., Lin, Y.C., Zheng, C.T., Zhang, H.X., Chen, W., Wang, S., Xia, W.G. and Li, Y., 2016. Effects of dietary methionine on performance, egg quality and glutathione redox system in egg-laying ducks. *British Poultry Science*, 57, 818–823 248

Jin, L.Z., Ho, Y.W., Abdullah, N. and Jalaludin, S., 1997. Probiotics in poultry: modes of action. *World’s Poultry Science Journal*, 53, 351–368 249

Li, W.F., Rajput, I.R., Xu, X., Li, Y.L., Lei, J., Huang, Q. and Wang, M.Q., 2011. Effects of probiotic (*Bacillus subtilis*) on laying performance, blood biochemical properties and intestinal microflora of Shaoxing duck. *International Journal of Poultry Science*, 10, 583–589 250

Mohan, B., Kadirvel, R., Bhaskaran, M. and Natarajan, A., 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. *British Poultry Science*, 36, 799–803 251

NRC, 1994. Nutrient requirements of poultry. National Research Council, (National Academy Press: Washington D.C) 252

Ooi, L.-G. and Liong, M.-T., 2010. Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings. *International Journal of Molecular Sciences*, 11, 2499–2522 253

Park, S., Kang, J., Choi, S., Park, H., Hwang, E., Kang, Y.G., Kim, A.R., Holzapfel, W. and Ji, Y., 2018. Cholesterol-lowering effect of *Lactobacillus rhamnosus* BFE5264 and its influence on the gut microbiome and propionate level in a murine model. 254

Patel, S.G., Raval, A.P., Bhagwat, S.R., Sadrasaniya, D.A., Patel, A.P. and Joshi, S.S., 2015. Effects of probiotics supplementation on growth performance, feed conversion ratio and economics of broilers. *Journal of Animal Research*, 5, 155 255

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing. (Vienna, Austria) 256

Ricke, S., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science*, 82, 632–639 257

Sandi, S., Yosi, F., Sari, M.L. and Gofar, N., 2018. The characteristics and potential of lactic acid bacteria as probiotics in silage made from *Hymenachne acutigluma* and *Neptunia oleracea* lour. In: E3S Web of Conferences. 258

Sandi, S., Yosi, F., Sari, M.L., Gofar, N. and Rofiq, M.N., 2020. Swamp forage silage organic acid profiles and influence of silage liquid organic acid salts against pathogenicbacteria in vitro. *International Journal of Poultry Science*, 19, 147–152 259

Soltan, M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood serum parameters in laying hens. *International Journal of Poultry Sciences*, 7, 613–621 260

Sudaryani, T., 2000. *Kualitas telur*, (Penebar Swadaya: Jakarta) 261

- 306 Sumiati, S., Darmawan, A. and Hermana, W., 2020. Performances and
307 egg quality of laying ducks fed diets containing cassava (*Manihot*
308 *esculenta* Crantz) leaf meal and golden snail (*Pomacea canalicu-*
309 *lata*). Tropical Animal Science Journal, 43, 227–232
- 310 Tomaro-Duchesneau, C., Jones, M.L., Shah, D., Jain, P., Saha, S. and
311 Prakash, S., 2014. Cholesterol assimilation by *Lactobacillus*
312 probiotic bacteria: An in vitro investigation. BioMed Research
313 International, 2014, 1–9
- 314 Zahri, I. and Febriansyah, A., 2014. Business diversification and its
315 impact on household income of farmers in swampy lowland areas.
316 Agricultural Socio-Economics Journal, 14, 144–153
- Ziaie, H., Bashtani, M., Torshizi, M.A.K., Naeemipour, H., Farhang-
far, H., Zeinali, A. and others, 2011. Effect of antibiotic and its
alternatives on morphometric characteristics, mineral content and
bone strength of tibia in Ross broiler chickens. Global Veterinaria,
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