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SHORT COMMUNICATIONS

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

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

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

Introduction

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

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

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

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

Materials and methods

Experimental animal and treatments

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

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

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

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

*Kcal/kg, calculated based onNRC (1994)

Laboratory analyses and measurement methods

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

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

Statistical analysis

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

Result and discussion

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

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

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

SEM standard error of mean

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

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

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

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

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

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

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

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

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Data availability On request.

Code availability Not applicable.

Declarations

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

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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