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Effect of Administering *Lactobacillus* Culture Isolated from Ensiled *Hymenache acutigluma* via Drinking Water on Meat and Egg Quality of Pegagan Ducks

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ABSTRACT

Lactobacillus spp. is one of the lactic acid bacteria, has a positive effect on improving the meat and egg quality in poultry. However, there is a paucity of information about the effect of *Lactobacillus* culture isolated from ensiled swamp grass on meat and egg characteristics. The present study aimed to investigate the effect of supplementing *Lactobacillus* culture isolated from ensiled *Hymenache acutigluma* (LHA) via drinking water on the duck meat and egg quality. A total of 60 Pegagan ducks aged 24-week-old were used in the current study and kept for 60 days. Ducks were randomly allocated into 5 treatment groups and 4 replicates per group, consisting of LHA 0 (control group without LHA solution), LHA 1, LHA 2, LHA 3, and LHA 4 (treatment groups supplemented with LHA solutions in drinking water with concentrations of 10^6, 10^7, 10^8, and 10^9 CFU/ml, respectively). The results showed that the value of cooking loss linearly increased, followed by a linear decrease in the water holding capacity after LHA addition. However, there was no change in the meat pH and moisture content. Further measurements revealed that both the yolk height and yolk index were linearly improved after being administered with LHA solutions, but no difference was found in other egg variables, including the yolk weight, diameter, and color, albumen weight, and height, as well as the eggshell weight and thickness. In conclusion, the provision of LHA via drinking water with a concentration of up to 10^9 CFU/ml could modulate the meat and egg quality of ducks. The LHA solutions enhanced the ability of meat protein to bind water, thereby inhibiting nutrient loss. Moreover, LHA had a greater effect on improving yolk quality, compared to albumen and eggshell.

Keywords: Drinking water, Egg quality, Ensiled *Hymenache acutigluma*, *Lactobacillus*, Meat, Pegagan ducks

INTRODUCTION

As one of the lactic acid bacteria, *Lactobacillus* spp. is the most commonly used probiotics for poultry and has a positive impact on the growth performance of ducks, such as increasing body weight and egg production, improving feed intake, and reducing feed conversion (Hassan and Komilus, 2020; Khattab et al., 2021). These benefits are supported by the main ability of *Lactobacillus* spp. in repressing the colonization and proliferation of various pathogenic bacteria in the digestive tract (Wang et al., 2018; Bae et al., 2020), thereby creating a healthy and optimally functioning intestine. Due to these benefits, many studies have been conducted on isolating *Lactobacillus* bacteria from various sources, such as the gastrointestinal tract (Kamollerd et al., 2016; Pokorná et al., 2019) and fermented foods or dairy products (Caggia et al., 2015; Rao et al., 2015; Xu et al., 2020). However, the use of *Lactobacillus* cultures isolated from ensiled swamp forage is still limited and has not been investigated so far.

In a previous study, several isolates derived from the silage of *Hymenache acutigluma*, which is one of the swamp forages that grow in the swamplands of South Sumatra, Indonesia, have been successfully cultured (Sandi et al., 2018). All isolates obtained from this ensilage belong to the genus *Lactobacillus* which is highly resistant to acidity conditions, both at low and high pH (Sandi et al., 2019). Based on the previous investigation, it was identified that the oral supplementation of *Lactobacillus* isolated from ensiled *Hymenache acutigluma* (LHA) has a positive outcome on improving the relative weight and length of internal organs and lowering lipids in duck serum (Yosi et al., 2020).

In addition to improving the growth performance of poultry, growing evidence has verified that administration of *Lactobacillus* strains could improve meat quality, such as maintaining pH, increasing water holding capacity, and reducing cooking loss (Wang et al., 2019; Dev et al., 2020). Some investigations also reported that not only meat quality but also egg quality could be modulated by the administration of *Lactobacillus* cultures, such as egg weight, yolk and albumen indices, shell thickness, Haugh unit, and yolk color (Gallazzi et al., 2008; Siadati et al., 2018). Since the relationship between LHA supplementation and poultry product quality has not been investigated so far, there is a need to conduct further examination addressing the effect of LHA on modulating meat and egg properties. As limited information is available on ducks, therefore, Pegagan ducks, known as a local duck from South Sumatra, Indonesia, were used in the current study

MATERIALS AND METHODS

Ethical approval

This experiment was performed in accordance with regulation 18/2015 on livestock, animal health and welfare in Indonesia, and ethical standards at Sriwijaya University, South Sumatra, Indonesia.

Experimental design

After an adaptation period of 3 days, a total of sixty 24-week-old Pegagan ducks, which was a local duck obtained from a duck farmer in Indralaya Regency, South Sumatra, Indonesia, were randomly distributed into 20 plots and reared for 60 days. The treatments consisted of 5 groups and 4 replicates for each group by supplementing LHA solutions with increasing concentrations, namely: LHA 0 (control group without LHA), LHA 1, LHA 2, LHA 3, and LHA 4 (LHA groups with a concentration of 10^6, 10^7, 10^8, and 10^9 CFU/ml, respectively). Determination of LHA concentration referred to the number of *Lactobacillus* occupying the gastrointestinal tract of broiler chickens, which ranged from 10^6 to 10^9 CFU/g contents (Rehman et al., 2007). The LHA solutions were delivered via drinking water during the first 30 days of the experimental period with an amount of 10 ml/bird/day. The diet was formulated to meet or exceed nutrients for egg-type ducks in the laying period according to Indonesian National Standard and administered *ad libitum* throughout the experiment. The composition of nutrients and ingredients of the experimental diet is presented in Table 1.

Preparation of ensiled *Hymenache acutigluma*

The process of making *Hymenache acutigluma* silage followed the procedure as described by the previous study (Yosi et al., 2020). The initial stage was cutting the fresh grass into smaller pieces of about 2-5 cm, followed by the withering process by storing it at 27-30°C and not being exposed to direct sunlight for at least 24 hours. A total of 500 g of withered grass was mixed with molasses and water (0.3% of the grass's dried weight), then put in a three-layer plastic bag under anaerobic conditions and stored at room temperature for 21 days.

Preparation of LHA solutions

The detailed stages of preparation of the LHA solutions and determination of their concentrations were as described by Yosi et al. (2020) with modification. *Lactobacillus* isolates were first incubated for 48 hours at 37°C, which had previously been cultured in deMan Rogosa Sharpe broth (Sandi et al., 2018). After that, the isolates were mixed with peptone solution replacing 0.85% NaCl solution, homogenized, and compared with McFarland standard solution to determine the concentration of LHA solutions based on the level of turbidity.

Table 1. Nutrient composition and ingredients of the experimental diet for Pegagan laying ducks for 2 months

Ingredients	Composition (%)
Corn meal	16
Dried noodle waste meal	40
Concentrate[a]	32
Bran	10
Premix[b]	1.0
Methionine	0.6
Lysine	0.4
Total	100
Calculated nutrient content[c]	
Metabolizable energy (Kcal/kg)	3007.2
Crude protein (%)	18.74
Crude fiber (%)	4.60
Calcium (%)	4.22
Available phosphorus (%)	0.46

[a]: A mixture of fish meal, soybean meal, coconut meal, meat and bone meal, wheat flakes, peanut meal, canola, leaf meal, vitamins, calcium, phosphate, and trace minerals. [b]: Provided per kilogram of diet, including Calcium (32.5%), Phosphor (1%), Iron (6 g), Manganese (4 g), Iodine (0.075 g), Copper (0.3 g), Zinc (3.75 g), Vitamin B12 (0.5 mg), and Vitamin D3 (50,000,000 IU). [c]: Calculated according to the recommendation of the Indonesian National Standard.

Meat quality analysis

After 2 months of rearing, one 32-week-old duck from each replicate was selected to measure meat quality, which included pH, water holding capacity (WHC), cooking loss (CL), and moisture content (MC). Measurement of pH, WHC, and CL followed the procedure as described by Yosi and Sandi (2014), while MC was analyzed according to the procedure of AOAC (2000). The pH value was observed 24 hours after slaughter, which was previously stored at - 4◦C (Lan et al., 2017). After being mashed with a meat grinder, 2 g of breast meat samples were weighed and dissolved in 18 ml distilled water until homogeneous. After completely dissolved, the solution was filtered and then measured using a pH meter (pHep Hanna 98107) that had previously been calibrated with standard solutions of 4 and 7. Before measuring CL, 20 g sample of breast meat was weighed and then put into polyethylene plastic. After being sealed with a vacuum pack, the samples were cooked in a water bath at 80^{o}C for 30 minutes and then cooled at room temperature (25-27^{o}C). The meat surface was dried with filter paper and its weight was measured using an analytical balance (Yosi and Sandi, 2014). Once recorded, the CL was calculated by dividing the weight loss during cooking by the weight of fresh meat and expressed in percentage. To measure MC, 1 g of breast meat was weighed and dried using an oven at 100–105°C until its weight was constant. For the WHC, 0.3 g of meat was first placed on Whatman 41 filter paper and then pressed between 2 metal plates with a load of 35 kg for 5 minutes. The wet area was determined by subtracting the total area with the area covered by the meat sample (Yosi and Sandi, 2014).

Egg quality measurements

After supplementing with LHA solutions in drinking water for 30 days, 2 eggs per replicate were randomly selected for measurements of the quality of yolk, albumen, and shell. The process of measuring egg variables was the same as described by Yosi et al. (2016). Each sample egg was weighed with a digital scale (Metler AE100-0.001) and recorded. The yolk and albumen were separated using an egg separator and weighed. The shell weight was calculated by subtracting yolk and albumen from the whole egg weight. Albumen and yolk height were determined with a tripod micrometer, while yolk diameter and albumen length and width were measured using a caliper. The shell thickness was calculated from the average measurement at the top, bottom, and center of the shell, which was measured using a micrometer screw gauge. Determination of yolk color was performed by comparing the Roche color fan with the color of each yolk. The yolk and albumen indices and Haught unit were calculated referring to Yosi et al. (2019) as follows: Yolk index = yolk height/yolk diameter, while Albumen index = albumen height/average of albumen length and width. The Haugh unit was obtained with the calculation: $HU = 100 \log (H + 7.57 - 1.7W^{0.37})$, where W and H are egg weight (g) and thick albumen height (mm), respectively.

Statistical analysis

All experimental data were analyzed using SPSS statistical software (IBM SPSS version 26). The data were subjected to one-way ANOVA. In case of a significant effect on the meat and egg quality parameters, Duncan's multiple comparison test was used to find out the differences between the treatment groups. In addition, the orthogonal comparison was performed with polynomial regression to determine the linear, quadratic, and cubic effects of increasing concentration of LHA supplementation via drinking water. After analysis, the data were presented in tables as means with pooled standard error of the mean (SEM). $P < 0.05$ was considered as a statistical difference.

RESULTS

Effects on meat quality

In the current study, meat quality covering pH, WHC, MC, and CL was measured and the results were presented in Table 2. The present findings revealed that the CL and WHC of breast meat were significantly influenced by treatments ($p < 0.05$), but no changes were identified in the meat pH and MC after consuming LHA in drinking water ($p > 0.05$). Furthermore, there was a linear decrease in meat CL and an increase in WHC at the same time after LHA addition. Both the control and LHA groups with a concentration up to 10^{8} CFU/ml did not exhibit any difference in the value of CL ($p > 0.05$). However, it then declined after the administration of LHA solution with a concentration of 10^{9} CFU/ml ($p < 0.05$). In contrast to WHC, where it started increasing after the addition of LHA with a concentration of 10^{7} CFU/ml compared to the control group ($p < 0.05$).

Effects on egg quality

Based on the analysis of the yolk parameters presented in Table 3, it was confirmed that simply the height of the yolk linearly improved with the LHA addition ($p < 0.05$), while no alterations were found in the weight, diameter, and color of the yolk. Insignificant differences between treatment groups were also identified in the albumen and eggshell parameters, including albumen and shell weight, albumen height, and shell thickness ($p > 0.05$). Furthermore, some calculations presented that LHA supplementation in drinking water noticeably elevated the yolk index ($p < 0.05$), but no effect ($p > 0.05$) was detected on the albumen index and Haugh unit of duck eggs (Graph 1). A significant improvement in yolk index occurred after the administration of LHA solution with a concentration of 10^8 CFU/ml and above in drinking water, compared to the control group ($p < 0.05$).

DISCUSSION

The value of pH plays a crucial role in meat quality (Yosi and Sandi, 2014). The non-significant influence on the breast meat pH has also occurred as reported by Chen et al. (2018) with applying *Lactobacillus rhamnosus* strain CF (Cheng Fu) in the White Leghorns chicken diet. Similarly, other investigations that apply different genus of bacteria, such as *Bacillus coagulans*, *Bacillus subtilis* fmbJ, and *Enterococcus faecium* also reported no different results on meat pH (Zhou et al., 2010; Bai et al., 2017; Lan et al., 2017). However, studies conducted either by Abdulla et al. (2017) or Atela et al. (2019) confirmed that the pH of the breast meat in broiler chickens markedly changed after supplementation with *Bacillus subtilis* DSM 17299 or multi-strain bacteria. These results showed that variations in using bacterial species/strains as probiotics or experimental design might be the main factor in determining the meat pH changes in poultry (Popova, 2017). Further, the meat MC after LHA supplementation was not different from that of the control. This finding is in line with studies by Abdulla et al. (2017) and Zhou et al. (2010) in which *Bacillus subtilis* DSM 17299 and *Bacillus coagulans* ZJU0616 were implemented as probiotics in feed, respectively, where the MC of breast muscle of chicken did not alter by treatment groups.

The WHC and CL are important indicators that are interrelated and commonly used in defining meat quality (Mohammed et al., 2021). A decrease in meat CL followed by improvement in WHC has a positive effect on meat quality (Yosi and Sandi, 2014). The present results were also in agreement with the study by Zheng et al. (2014) who reported that the administration of *Enterococcus faecium* notably improved WHC and reduced CL in the broiler chickens' breast meat. Likewise, Abou-Kassem et al. (2021) also recorded that there was a decrease in the CL and increase in WHC in meats of 42-day growing Japanese quails after supplementing *Bacillus toyonensi* and *Bifidobacterium bifidum* in the diet. It was verified that the improved meat WHC after probiotic addition in the diet was highly regulated by the presence of muscle protein (Zheng et al., 2014), pointing that the administering of LHA also might have a major effect on the abundance of meat proteins which then contribute to enhanced water holding capacity in duck meat. In addition, it is also presumed that LHA might affect the intramuscular fat content and polyunsaturated fatty acids composition of duck's breast meat. According to the previous study, the improved meat quality after consuming *Clostridium butyricum* was associated with the changes in the meat fatty acid composition, such as n-3 and n-6 polyunsaturated fatty acids (Yang et al., 2010). However, this assumption needs to be examined through further investigation by measuring the fatty acid composition in the duck meat.

Table 2. Meat quality of Pegagan laying ducks after consuming *Lactobacillus* isolated from ensiled *Hymenache acutigluma* via drinking water for 30 days

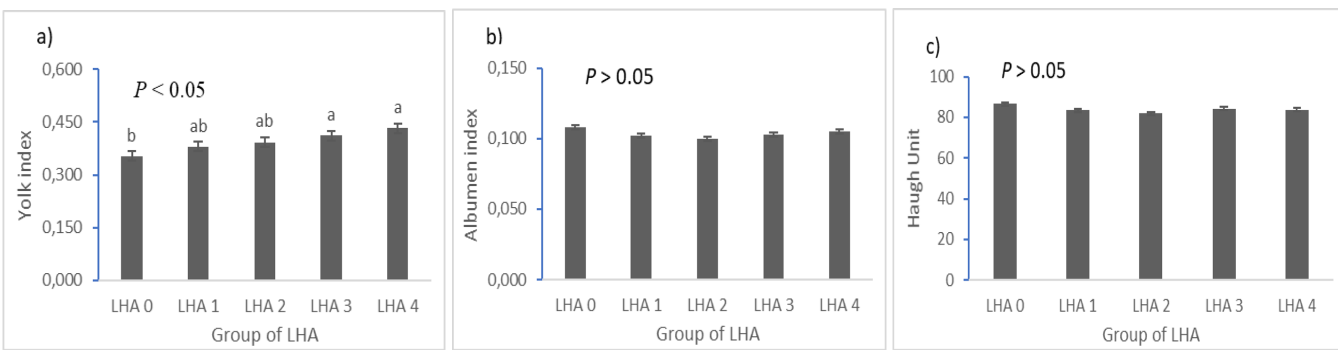
Item	Groups of LHA					SEM	p value		
	LHA 0	LHA 1	LHA 2	LHA 3	LHA 4		Linear	Quadratic	Cubic
pH	5.73	5.63	5.65	5.75	5.70	0.09	0.721	0.484	0.202
Cooking loss (%)	32.79^{a}	33.86^{a}	30.88^{ab}	31.7^{ab}	28.78^{b}	1.48	0.008	0.304	0.922
Moisture content (%)	74.63	74.83	76.07	74.23	75.80	0.78	0.338	0.869	0.193
Water holding capacity (%)	37.58^{b}	40.81^{ab}	43.13^{a}	43.49^{a}	42.84^{a}	1.77	0.005	0.057	0.980

LHA: *Lactobacillus* solutions isolated from ensiled *Hymenache acutigluma*, SEM: Standard error of the mean, ^{a,b} different superscripts in the same row mean significant difference ($p < 0.05$). LHA 0: Control group, LHA 1, 2, 3, and 4: LHA groups with a concentration of 10^6, 10^7, 10^8, and 10^9 CFU/ml, respectively.

Table 3. Egg quality of Pegagan laying ducks after consuming *Lactobacillus* isolated from ensiled *Hymenache acutigluma* via drinking water for 30 days

Item	Groups of LHA					SEM	p value		
	LHA 0	LHA 1	LHA 2	LHA 3	LHA 4		Linear	Quadratic	Cubic
Yolk									
Weight (g)	20.97	20.63	20.49	20.35	20.41	0.55	0.265	0.593	0.997
Diameter (cm)	4.53	4.36	4.54	4.18	4.36	0.15	0.146	0.687	0.535
Height (cm)	1.58^{b}	1.65^{ab}	1.78^{ab}	1.73^{ab}	1.88^{a}	0.11	0.009	1.000	0.499
Color	13.25	14.00	13.50	14.00	13.75	0.28	0.142	0.210	0.451
Albumen									
Weight (g)	39.93	41.39	43.28	39.92	37.52	2.34	0.248	0.054	0.919
Height (cm)	0.78	0.75	0.73	0.74	0.73	0.06	0.384	0.680	0.845
Shell									
Weight (g)	4.43	4.60	4.81	4.43	4.17	0.22	0.183	0.068	0.895
Thickness (mm)	0.36	0.39	0.34	0.38	0.37	0.03	0.808	0.791	0.580

LHA: *Lactobacillus* solutions isolated from ensiled *Hymenache acutigluma*, SEM: Standard error of mean, ^{a,b} different superscripts in the same row mean significant difference between treatment groups ($p < 0.05$). LHA 0: Control group, LHA 1, 2, 3, and 4: LHA groups with a concentration of 10^6, 10^7, 10^8 and 10^9 CFU/ml, respectively.



Graph 1. Yolk index (a), albumen index (b), and Haugh unit (c) of Pegagan duck after supplementing with *Lactobacillus* isolated from ensiled *Hymenache acutigluma* (LHA) via drinking water for 30 days. LHA 0: Control group, LHA 1, 2, 3, and 4: LHA groups with a concentration of 10^6, 10^7, $10^{8,}$ and 10^9 CFU/ml, respectively. The different letters (a, b) above the column mean significant difference ($p < 0.05$).

The internal and external quality of eggs can be evaluated from the parameters of the yolk, albumen, and eggshell properties (Rath et al., 2015). After administering LHA solutions via drinking water for 1 month, most of the egg parameters measured in this study were not altered, except the yolk height and index were notably improved. Kalavathy et al. (2005) and Siadati et al. (2018) also reported that no improvement was recorded in yolk parameters, including yolk color and weight, after applying *Lactobacillus* strains either in the laying hens or quails diet. A slightly different outcome was presented by Forte et al. (2016), where there was a meaningful change in the yolk color after being enriched with *Lactobacillus acidophilus* and *Bacillus subtilis* in the diet of laying hen.

Concerning the physical quality of albumen, several studies recorded that there were variations in poultry responses after *Lactobacillus* supplementation. The insignificant results of albumen parameters in this study were also in compliance with the investigation by Saksrithai and King (2020), confirming that albumen height and Haugh unit of laying hens aged 58 to 60 weeks did not differ between control and treatment groups with adding *Lactobacillus* culture into drinking water. Similarly, Forte et al. (2016) also reported that laying hens supplied with dietary *Lactobacillus acidophilus* and *Bacillus subtilis* exhibited no change in the weight and percentage of albumen and Haugh units. On the opposite, some studies have demonstrated a positive effect of supplementing *Lactobacillus* in enhancing albumen quality. Gallazzi et al. (2008) affirmed that the application of *Lactobacillus acidophilus* D2/CSL as probiotics was proven to increase Haugh unit in laying hens. This is also in line with Siadati et al. (2018) that the provision of native *Lactobacillus* strains had positive effects on albumen height and the Haugh unit in Japanese quails. For eggshell parameters, the results from the current study are also supported by other studies, where no effect was obtained either on shell weight or shell thickness by the inclusion of *Lactobacillus acidophilus* D2/CSL or *Enterococcus faecalis* (Gallazzi et al., 2008; Zhang et al., 2019). In reverse, a study by Panda et al. (2008) identified a significant increase in shell thickness after consuming *Lactobacillus sporogenes* in the feed. Besides *Lactobacillus*, the provision of *Pediococcus acidilactici* also provided beneficial outcomes for increasing eggshell thickness in laying hens (Mikulski et al., 2012). Based on this observation, it is assumed that the varied effects of bacteria as probiotics on egg quality might be influenced by the type and the administration method of the bacteria.

CONCLUSION

In conclusion, the provision of *Lactobacillus* culture isolated from ensiled *Hymenache acutigluma* (LHA) via drinking water with a concentration of up to 10^9 CFU/ml could modulate the meat and egg quality of Pegagan ducks. The LHA solutions might enhance the ability of meat protein to bind to water, thereby inhibiting nutrient loss. Moreover, LHA had a greater effect on improving yolk quality compared to albumen and eggshell.

DECLARATIONS

Competing interests

All authors involved in this research work declare having no competing interests

Authors' contributions

FY conceptualized the research work, collected and analyzed the sample, processed and interpreted the data, and wrote the initial manuscript. NG and SS participated in drafting the research plan. ES and MLS were involved in the analysis and interpretation of the data. FF and HY conducted animal trials, collected and analyzed samples in the laboratory. All authors revised and approved the final version of the manuscript.

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Ethical considerations

All related to ethical issues, consisting of plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by the authors.

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