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Supplementation of Lactic Acid Bacteria Derived from Ensiled Kumpai Tembaga on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks

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Abstract | Lactic acid bacteria (LAB) are a very potential candidate as probiotics that provide health benefits to the host by improving the intestine microbial balance. This study was performed to investigate the influence concentration of LAB derived from Kumpai Tembaga silage on live body weight, the length and relative weight of the gastrointestinal tract and internal organs, and blood characteristics in Pegagan ducks. One hundred of 7-day-old Pegagan ducks were randomly divided into 5 group treatments and 4 replicates: the first treatment was the control (without LAB), the second to the fifth treatment was LAB supplementation with a concentration of $1x10^6$, 10^7, 10^8, and 10^9 cfu/ml, respectively. Samples were collected at 8 weeks of life to determine the live body weight, length and relative weight of the gastrointestinal tract and internal organs, and hematological and serum biochemical parameters. The administration of LAB with various concentrations improved the live body weight and increased the length and relative weight of the total small intestine, duodenum, jejunum, and caeca. Moreover, LAB supplementation also has a positive effect on lowering the serum level of cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), where the higher concentration of LAB resulted in the greater decrease in serum lipids. It can be concluded that the potential of LAB derived from Kumpai Tembaga silage by providing concentrations up to 10^9 cfu/ml is very considerable, particularly in improving the body weight, enhancing the digestive function, and reducing serum lipid levels in Pegagan duck.

Keywords | Blood profile, Gastrointestinal tract, Kumpai Tembaga silage, Lactic acid bacteria, Pegagan ducks

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INTRODUCTION

Since the use of dietary antibiotics as antimicrobial growth promoters (AGPs) has negative impacts on animal and human health (Ghasemi-Sadabadi et al., 2019), many countries across the world have strictly prohibited the use of these antibiotics in the poultry industry (Youssef et al., 2017). These health threats arise due to the increased resistance of pathogenic bacteria to antibiotics and the accumulation of antibiotic residues in poultry products (Park et al., 2018). This difficult situation encouraged studies to discover new alternative additives and eventually emerged probiotics as antibiotic replacements (Chen et al., 2013; Çalik et al., 2017). Probiotics or direct-fed microbials are defined as living microorganisms that provide health benefits to the host by improving the intestine microbial balance (Reis et al., 2017). Probiotics are very effective in improving the intestinal microbial balance (Song et al., 2014), suppressing the proliferation of pathogenic bacteria in the digestive tract (Park and Kim, 2014), increasing the body antioxidant levels (Bai et al., 2017), and enhancing intestinal immunity (Bai et al., 2013). The improved growth

performances in poultry by administering probiotics, such as increasing body weight gain, improving egg production, and elevating the relative weight of internal organs, are also well documented by many studies (Park and Kim, 2014; Balamuralikrishnan et al., 2017; Upadhaya et al., 2019)

In many studies, lactic acid bacteria (LAB) are a very potential candidate as probiotics because they have specific characteristics, such as high tolerance to gastrointestinal conditions, having cellulolytic activity, producing undissociated volatile fatty acids, high ability to attach in the intestinal epithelium, reducing colonization of pathogenic bacteria, and resistant to the bile salts influence (Kim et al., 2015; Shokryazdan et al., 2017; Al-Khalaifah, 2018; Herdian et al., 2018; Martin et al., 2018; Pokorná et al., 2019). There are several genera of LAB that are widely used probiotics in poultry, including *Lactobacillus* (Mermouri et al., 2017; Ahmed et al., 2019), *Enterococcus* (Royan, 2018) and *Bifidobacterium* (Al-Khalaifa et al., 2019). The LAB probiotics are also able to improve both the physiological status and growth performance in poultry (Lan et al., 2017; Al-Khalaifah, 2018), such as increasing the weight gain, the relative weight of internal organs, and immune response. In recent years, studies have been performed by isolating LAB from traditional fermented foods and products such as coconut palm inflorescence or Neera (Somashekaraiah et al., 2019), cheese (Hashemi et al., 2014; Caggia et al., 2015), fermented cereal-based foods (Adesulu-Dahunsi et al., 2018), and kimchi (Kim et al., 2015). In addition, LAB probiotics are also isolated from the gastrointestinal segments in poultry (Martin et al., 2018; Aziz et al., 2019; Shi et al., 2020),such as colon, bile, and caecum.

Our team has developed a study regarding the identification of LAB isolated from Kumpai Tembaga silage (Sandi et al., 2018). The Kumpai Tembaga is the local name for the *Hymenachne acutigluma* grass that can be easily obtained from the swamp area in South Sumatra, Indonesia. Our findings revealed that the LAB isolated from Kumpai Tembaga silage belongs to the *Lactobacillus* group. Based on in vitro, the identified LAB has high acid resistance and is able to adapt to low (3-6.5) and high (7.5-8) pH (Sandi et al., 2019). It is assumed that the concentration and the strains of bacteria are the crucial factors to be considered in achieving optimal growth performance. A study showed that administering *Bacillus subtilis* UBT-MO2 with a concentration of 10^5 cfu is able to improve the growth performance and relative weight of internal organs in poultry (Zhang et al., 2013). Meanwhile, another study reported that optimal growth was obtained with the use of *Bacillus subtilis* of 10^8 cfu (Zhang et al., 2012). Therefore, this in vivo study aims to investigate the influence concentrations of LAB derived from Kumpai Tembaga silage on live body weight, the length and relative weight of the gastrointestinal tract and internal organs, and blood characteristics in Pegagan ducks.

MATERIALS AND METHODS

Birds, diets and experimental design

All procedures conducted in this study involving Pegagan ducks were in accordance with the ethical standards of the Sriwijaya University and also the regulation of the Republic of Indonesia No. 18 in 2009 regarding animal farming, health, and welfare. A total of 100 unsexed 7-day-old Pegagan ducks, with average body weight (BW) of 115.31 ± 5.40 g, were obtained from a duck farming located in Ogan Ilir, South Sumatra. All ducks were weighed and randomly allocated to 5 experimental LAB groups with 4 replicate plots (100 x 75 cm) consisting of 5 birds each. Ducks were reared in an open sidewall housing for 7 weeks. The starter and finisher diets were based on corn-soybean meal and offered to the ducks starting from 0-2 and 2-8 weeks of life, respectively (Table 1). Diets were formulated to meet or exceed the nutrients recommendation by NRC (1994). Each pen was equipped with a manual plastic round feeder and drinker. Drinking water and diets were provided ad libitum. The LAB concentration treatments were as follows: P0 (control; without LAB); P1 (LAB of 1×10^6 cfu/ml); P2 (LAB of 1×10^7 cfu/ml), P3 (LAB of 1×10^8 cfu/ml), and P4 (LAB of 1×10^9 cfu/ml). The LAB was offered orally and gradually adjusted to the beak size. In the first 3 weeks of age, ducks were provided LAB of 3 ml/bird. Afterward, birds were administrated with LAB as many as 5, 7.5, and 10 ml at the age of 3-5, 5-7, and 7-8 weeks, respectively.

The making of kumpai tembaga silage

The making process of Kumpai Tembaga silage refers to our previous study (Sandi et al., 2018). Briefly, Kumpai Tembaga grass was cut about 2-5 cm and then stored for 24 h for the withering process. A total of 500 g of the withered grass was dissolved with a mixture of molasses and water as much as 3% of the grass weight. The dissolved grass was next put in 3 layers of plastic bags, sealed to anaerobic conditions, and stored for 21 days before being analyzed in the laboratory.

The LAB isolation and determination of the LAB concentration

In this study, The LAB were isolated from the Kumpai Tembaga (*Hymenachne acutigluma*) silage. The detailed steps for LAB isolation have been described by our previous study (Sandi et al., 2018). In brief, LAB isolates were cultured on media de man rogosa sharpe broth (MRS Broth) and incubated for 48 h at 37°C. Furthermore, the cultured LAB isolates were diluted with 0.85% NaCl solution. The determination of LAB concentration was by comparing the diluted LAB solution and the McFarland standard solution based on the level of turbidity.

Table 1: Ingredients and nutrient composition of the treatment diets (g/kg diet as fed basis).

Ingredients	Composition (%)	
	starter (0-2 wk)	finisher (2-8 wk)
Corn meal	56	68
Soybean meal	28	16
Bran	9	10
Meat Bone Meal (MBM)	6	5
Vitamin-mineral Premix[a]	0.5	0.5
Grit	0.5	0.5
Calculated chemichal composition[b]		
ME (Kcal/kg)	2910	3109
Crude protein (%)	22.06	18.16
Crude fiber (%)	6.24	7.96
Ca (%)	0.99	0.85
Available P (%)	0.67	0.52

[a]provided per kilogram of diet: methionine, 3,400 mg; lysine HCL, 5,000 mg, vitamin A, 5,000,0000 IU; vitamin D3, 1,500,000 IU; vitamin E, 450 IU; vitamin B2,1,500 mg; vitamin B6, 780 mg; vitamin B12, 3,800 mg; vitamin K, 1,500 mg; vitamin C, 330 mg; niacin, 5,580 mg; pantotenate acid, 1,800 mg; zinc sulphate, 4,000 mg; cooper, 4,000 mg; magnesium, 4,000 mg; sodium chloride, 16,500 mg; sodium sulphate, 70,0000 mg; potasium chloride, 29,000 mg; manganese, 4,000 mg. [b]Calculated according to ingredients composition provided by National Research Council (1994).

Measurement the weight of the live body, gastrointestinal tract and internal organs

At the end of the experiment, all ducks were weighed to determine the live body weight. The measurement of the relative weight and length of the gastrointestinal tract (GIT) and internal organs (IO) refers to Yosi et al. (2017). As many 2 birds in each treatment were randomly selected. Ducks were fasted and only provided drinking water for 8 h before slaughtering. The GIT contents were removed after being cut into each segment. The duodenal length was determined from the end of the gizzard outlet to the end of the pancreatic loop. Next, the length of jejunum was measured from the tip of the pancreatic loop to the Meckel's diverticulum, while the ileum length was measured from Meckel's diverticulum to the base of the cecal junction. The relative weight of the GIT and IO was calculated by dividing the weight of GIT segments or IO and the live body weight then multiplied by 100.

Blood hematological and serum biochemical measurements

Measurement of blood hematological and serum biochemical parameters according to Yosi et al. (2017). At the end of the experiment, as many 3 ml of venous blood samples from 2 birds per pen were collected by puncture of the brachial vein using sterilized syringes containing anticoagulant. The syringes were then capped and carried to the laboratory for counting the number of red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). While for biochemical analysis, blood samples were put into the tubes containing no anticoagulant and centrifuged at 3.220 × g for 8 min at 4°C. Serum was taken and stored at -20°C for analyzing of triglyceride, cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL), total protein, albumin, and globulin using enzymatic colorimetric methods.

Statistical analysis

Data were analyzed with a one-way ANOVA procedure using the SPSS software version 17. Data were displayed as means. Differences among means were examined using Duncan's multiple range tests. A test α level of $P < 0.05$ was applied to define statistical significance

RESULTS AND DISCUSSION

Live body weight of pegagan ducks

Based on our findings as shown in Table 2, the live body weight of Pegagan ducks was considerably ($p<0.05$) affected by LAB treatment. According to the concentration level of LAB, a notable effect ($p<0.05$) on body weight occurred when ducks were administered LAB starting at 10^7 cfu/ml and above compared to control treatment. The heightened body weight in this study was in line with the other studies (Shokryazdan et al., 2017; Abdel-Hafeez et al., 2017; Park et al., 2018), who stated that supplementation of probiotics was able to increase body weight gain and gain a greater body weight compared to the non-probiotic treatment in the whole experiment. These findings are also in agreement with Mohammadi Gheisar et al. (2016) and Lan et al. (2017) that dietary LAB probiotics containing *Enterococcus faecium* were able to improve the live body weight of chickens compared with the control treatment. The favorable effects of LAB in increasing body weight indicate that there are an enhanced intestinal digestive enzyme activity and improved nutrients digestion and absorption in the gastrointestinal tract (Mohammadi Gheisar et al., 2016; Park et al., 2016). According to Chen et al. (2013), the activity of digestive enzymes covering protease, amylase, and lipase was enhanced by the role of probiotics, hence optimizing digestion and uptake of nutrients in the gastrointestinal tract. This explanation is also confirmed by Wang and Gu (2010), that bacteria, primarily those belonging to the *Bacillus* genus, are capable of secreting exoenzymes and might stimulate the production of endogenous enzymes synthesized by the digestive tract

of poultry (amylase, protease, and lipase). In this study, a meaningful increase in live body weight happened when ducks consumed LAB starting at 10^7 cfu/ml. However, a different result presented by Wang and Gu (2010) that the administration of probiotic *B. coagulans* NJ0516 of 10^6 cfu/g via basal diet was able to significantly increase the final body weight of broilers. With an equal age at 8 weeks, the final live body weight of ducks obtained in this study was slightly lower compared to the body weight reported by Bidura et al. (2019) who was experimenting with the provision of probiotics containing *Saccharomyces* spp. KB-5, *Saccharomyces* spp. KB-8 or the recombination, which was 1.46–1.51 kg, whereas in this study the values were ranged from 1.17 to 1.37 kg. In this regard, differences in strains of probiotics have a major effect on the response to body weight gain (Khan et al., 2013).

The length and relative weight of the gastrointestinal tract and internal organs

Another significant result ($p<0.05$) was noted in the relative weight and length of gastrointestinal segments, among others total small intestine, duodenum, jejunum, and ceca. While for crop-esophagus, proventriculus, ileum, and colon, it presented an unmarked effect ($p>0.05$) on [illegible] weight and length (Table 2). Insignificant results ($p>0.05$) were also recorded in the relative weight of gizzard, liver, heart, spleen, pancreas, and bile (Table 3). The increased relative weight of small intestine, jejunum and cecum occurred when ducks were supplemented with LAB of 10^8 cfu/ml, except for the duodenum which was beginning to increase at 10^6 cfu/ml. While the length of the small intestine and ceca, a significant improvement ($p<0.05$) occurred after providing LAB of 10^6 cfu/ml. It is assumed that probiotics supplementation in this study has been able to enhance the metabolic rate and ultimately increase the relative weight and size of gastrointestinal parts, particularly in the small intestine (Abdel-Hafeez et al., 2017). Many studies associated with the administration of probiotics also documented significant and insignificant results on the weight of the digestive tract and internal organs. Comparable to our findings, Park and Kim (2014) reported that the relative weights of some internal organs were not changed by the administration of *B. subtilis* B2A with concentrations of 10^4-10^6 cfu. This result was also supported by Balamuralikrishnan et al. (2017) that the provision of probiotics, including the *Bacillus* and *Clostridium* genus of 10^8 and 10^9 cfu/g, did not show a significant impact on the weight of gizzard and other internal organs. In addition, the increased relative length of jejunum was also conferred by Reis et al. (2017) with the supplementation of probiotics of *B. subtilis* in broiler chicken's diet. The greater relative weight and length of the small intestine and caeca might be influenced by probiotic activity that improves intestinal morphology, such as villus height and crypt depth. This is also confirmed by other studies that the administration of probiotics was able to increase the villus height and villus height-to-crypt depth ratio in the small intestine of broiler (Sen et al., 2012; Lei et al., 2015; Agboola et al., 2015). The higher villus height will lead to the enlarged intestinal surface area (Tang et al., 2017), which has the potential to improve the relative weight and length of the small intestine. Furthermore, Hossain et al. (2015) stated that increased villus height and villus height-to-crypt depth ratio are directly related to enhanced epithelial turnover and longer villi were associated with activation of cell mitosis. In contrast to our findings, Abdel-Hafeez et al. (2017) noticed that probiotics did not significantly affect the relative weight of the small intestine (2.61%) in chickens at the end of the finisher period. A reverse result was also reported by Reis et al. (2017) that birds supplemented with *B. subtilis* definitely presented a reduced relative duodenum length. On the other hand, Aalaei et al. (2018) also reported that none of the jejunal morphological parameters changes in broilers supplemented with probiotics. It can be considered that variations in the strains, sources, viability, and concentrations of bacteria, and methods of administration might be the main factors causing different responses in poultry gastrointestinal tract.

Blood hematological parameters

According to hematological analysis, there were no significant differences ($p>0.05$) between the LAB supplementation and control groups in Hb, RBC, WBC, thrombocytes, PCV, MCV, MCH, and MCHC, but all of these parameters were within the normal ranges (Table 4). These insignificant results indicate that the concentration of LAB derived from Kumpai Tembaga silage was not been able to influence blood hematological values. The unmarked hematological parameters in this study are in line with other studies related to probiotic supplementation. The numbers of RBC and WBC in birds was reported not to be significantly increased by the administration of various probiotic strains, such as *Bacillus subtilis* RX7 and B2A (Park and Kim, 2014), *E. faecium* (Lan et al., 2017), and *B. subtilis* RX7 and C14 (Park et al., 2018), with the amounts of 2.00-2.01 (10^6/µL) and 27.7-28.5 (10^3/µL), 2.11-2.46 (10^6/µL) and 19.9- 20.8 (10^3/µL), and 2.17-2.22 (10^6/µL) and 29.2-31.0 (10^3/µL), respectively. Those RBC and WBC values appear to be lower than that of this study, namely 4.20-4.50 (10^6/µL) and 26.04-29.00 (10^3/µL). Additionally, the level of Hb, which is essential in oxygen transport, was also not significantly different between control and probiotics supplementation groups (Alkhalf et al., 2010; Khan et al., 2013). Based on sex differentiation, there was also no notable effect of administrating probiotics to the RBC, WBC, and Hb counts in broiler chicken male and

Table 2: Live body weight and the length and relative weight of gastrointestinal tract in Pegagan ducks fed different concentration of LAB derived from Kumpai Tembaga silage.

Traits	Concentration of LAB				
	P0	P1	P2	P3	P4
LBW (kg)	1.17 ± 0.06^{a}	1.26 ± 0.05^{ab}	1.28 ± 0.09^{ab}	1.30 ± 0.06^{b}	1.37 ± 0.10^{b}
GIW (%)					
Crop-oesofagus	0.63 ± 0.09	0.64 ± 0.06	0.62 ± 0.11	0.61 ± 0.14	0.60 ± 0.01
Proventiculus	4.73 ± 0.80	4.90 ± 0.19	4.61 ± 1.17	4.80 ± 0.85	4.69 ± 0.22
Small Intestine	1.99 ± 0.06^{a}	2.14 ± 0.09^{ab}	2.17 ± 0.19^{ab}	2.29 ± 0.17^{b}	2.32 ± 0.09^{b}
Duodenum	0.51 ± 0.02^{a}	0.57 ± 0.02^{b}	0.59 ± 0.06^{b}	0.57 ± 0.03^{b}	0.59 ± 0.03^{b}
Jejunum	0.67 ± 0.04^{a}	0.71 ± 0.06^{a}	0.74 ± 0.09^{ab}	0.82 ± 0.03^{b}	0.84 ± 0.09^{b}
Ileum	0.82 ± 0.06	0.86 ± 0.06	0.84 ± 0.08	0.90 ± 0.18	0.89 ± 0.04
Caeca	0.22 ± 0.04^{a}	0.21 ± 0.03^{a}	0.26 ± 0.03^{ab}	0.31 ± 0.04^{b}	0.30 ± 0.06^{b}
Colon	0.15 ± 0.05	0.18 ± 0.01	0.19 ± 0.03	0.20 ± 0.02	0.19 ± 0.05
GIL (cm)					
Crop-oesofagus	20.68 ± 0.96	22.10 ± 1.58	21.60 ± 1.42	21.93 ± 2.65	23.68 ± 3.59
Small Intestine	141.60 ± 4.32^{a}	158.98 ± 6.50^{b}	158.88 ± 7.33^{b}	160.40 ± 10.15^{b}	163.80 ± 8.17^{b}
Duodenum	33.80 ± 3.99^{a}	38.58 ± 1.09^{b}	38.93 ± 0.94^{b}	39.35 ± 3.28^{b}	39.78 ± 2.49^{b}
Jejunum	50.23 ± 3.48^{a}	59.13 ± 3.35^{b}	58.85 ± 4.09^{b}	58.05 ± 2.88^{b}	59.28 ± 6.10^{b}
Ileum	57.58 ± 2.05	61.28 ± 3.42	61.10 ± 5.35	63.00 ± 4.39	64.75 ± 4.56
Caeca	13.68 ± 0.46^{a}	14.88 ± 0.82^{b}	14.90 ± 0.42^{b}	14.70 ± 0.39^{b}	14.98 ± 0.74^{b}
Colon	9.23 ± 1.34	9.08 ± 0.94	9.90 ± 1.15	9.95 ± 1.27	9.85 ± 1.69

$^{a-b}$Means within a row with no common superscript differ significantly (P<0.05). LBW: live body weight; GIW: gastrointestinal relative weight; GIL: gastrointestinal length; P0: control; without LAB, P1: LAB of 1×10^{6} cfu/ml, P2: LAB of 1×10^{7} cfu/ml, P3: LAB of 1×10^{8} cfu/ml, and P4: LAB of 1×10^{9} cfu/ml.

Table 3: The relative weight of internal organs in Pegagan ducks fed different concentration of LAB derived from Kumpai Tembaga silage.

Traits	Concentration of LAB				
	P0	P1	P2	P3	P4
IO (%)					
Gizzard	3.92 ± 0.26	4.01 ± 0.24	4.08 ± 0.44	3.89 ± 0.40	3.95 ± 0.12
Heart	0.70 ± 0.09	0.73 ± 0.10	0.73 ± 0.07	0.71 ± 0.09	0.70 ± 0.08
Liver	2.35 ± 0.21	2.26 ± 0.34	2.28 ± 0.24	2.31 ± 0.08	2.33 ± 0.15
Spleen	0.13 ± 0.02	0.12 ± 0.02	0.14 ± 0.03	0.14 ± 0.02	0.12 ± 0.02
Pancreas	0.82 ± 0.07	0.86 ± 0.06	0.84 ± 0.08	0.89 ± 0.17	0.88 ± 0.04
Bile	0.30 ± 0.07	0.30 ± 0.04	0.31 ± 0.05	0.29 ± 0.04	0.30 ± 0.06

IO: Internal organs; P0: control; without LAB, P1: LAB of 1×10^{6} cfu/ml, P2: LAB of 1×10^{7} cfu/ml, P3: LAB of 1×10^{8} cfu/ml, and P4: LAB of 1×10^{9} cfu/ml.

female aged 42 d of life (Ghasemi-Sadabadi et al., 2019). Probiotics might be able to improve the acidic conditions in the digestive tract induced by the fermentation process, which conclusively can absorb more iron for the formation of blood hemoglobin (Abdel-Hafeez et al., 2017). The insignificant influence of probiotics on thrombocyte count and other haemogram parameters, including MCH, MCV, MCHC, and PCV, was also reported by Tang et al. (2017), which was using probiotic PrimaLac on observed laying hens at 36 and 52 wk of life. This is also in line with Al-Khalaifa et al. (2019) on 5-wk-broiler chickens supplemented with probiotics of *Bacillus* and *Lactobacillus*.

Serum biochemical parameters

The administration of LAB significantly influenced ($p<0.05$) the serum level of cholesterol, triglycerides, HDL, and LDL of Pegagan ducks. However, the level of total protein, albumin, and globulin in serum was not affected ($p>0.05$) by LAB concentration treatments (Table 5). Further, ducks fed the higher level of LAB resulted in a

Table 4: Blood hematological parameters in Pegagan ducks fed different concentration of LAB derived from Kumpai Tembaga silage.

Traits	Concentration of LAB				
	P0	P1	P2	P3	P4
Hb (g/dl)	13.75 ± 0.98	13.40 ± 0.58	13.25 ± 1.56	13.45 ± 1.33	12.60 ± 0.35
WBC (10^3/µL)	26.64 ± 2.96	26.04 ± 2.26	28.04 ± 3.09	29.00 ± 1.15	28.60 ± 1.85
RBC (10^6/µL)	4.75 ± 0.52	4.45 ± 0.17	4.45 ± 0.51	4.50 ± 0.46	4.20 ± 0.23
PCV (%)	41.0 ± 4.62	41.50 ± 1.73	41.0 ± 4.62	41.5 ± 4.04	39.0 ± 2.31
Thrombocyte (10^3/µL)	45.20 ± 0.92	44.40 ± 3.35	46.45 ± 2.14	49.40 ± 0.69	49.00 ± 2.31
MCV (fl)	93.25 ± 0.26	92.86 ± 0.12	92.67 ± 0.20	92.26 ± 0.48	92.66 ± 0.41
MCH (pg)	30.39 ± 0.45	30.11 ± 0.13	29.77 ± 0.02	29.90 ± 0.12	30.00 ± 0.31
MCHC (%)	32.38 ± 0.14	32.29 ± 0.05	32.30 ± 0.16	32.41 ± 0.04	32.31 ± 0.03

Hb: hemoglobin; WBC: white blood cell; RBC: red blood cell; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: meancorpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration. P0: control; without LAB, P1: LAB of 1×10^6 cfu/ml, P2: LAB of 1×10^7 cfu/ml, P3: LAB of 1×10^8 cfu/ml and P4: LAB of 1×10^9 cfu/ml.

Table 5: Serum biochemical parameters of Pegagan ducks fed different concentration of LAB derived from Kumpai Tembaga silage.

Traits	Concentration of LAB				
	P0	P1	P2	P3	P4
Cholesterol (mg/dL)	180.5 ± 2.89^{d}	174.5 ± 5.20^{e}	172.5 ± 4.04^{c}	156.0 ± 4.62^{b}	131.5 ± 7.51^{a}
Triglyicerides (mg/dL)	108.0 ± 3.46^{b}	110.5 ± 2.89^{b}	105.5 ± 4.04^{ab}	100.0 ± 2.31^{a}	101.0 ± 5.77^{a}
HDL (mg/dL)	57.5 ± 2.89^{b}	53.5 ± 0.58^{a}	51.0 ± 3.46^{a}	50.5 ± 1.73^{a}	51.5 ± 1.73^{a}
LDL (mg/dL)	131.0 ± 6.93^{bc}	133.5 ± 7.51^{c}	121.5 ± 4.05^{ab}	121.0 ± 8.08^{a}	122.5 ± 1.73^{ab}
Total Protein (g/dL)	4.40 ± 0.20	4.11 ± 0.18	4.19 ± 0.66	4.13 ± 0.14	4.18 ± 0.03
Albumin (g/dL)	1.28 ± 0.08	1.13 ± 0.24	1.08 ± 0.12	1.03 ± 0.05	1.10 ± 0.02
Globulin (g/dL)	3.13 ± 0.12	3.01 ± 0.09	3.22 ± 0.12	3.11 ± 0.09	3.15 ± 0.08

[a-c]Means within a row with no common superscript differ significantly (P<0.05). LDL: low-density lipoprotein; HDL: high-density lipoprotein; P0: control; without LAB, P1: LAB of 1×10^6 cfu/ml, P2: LAB of 1×10^7 cfu/ml, P3: LAB of 1×10^8 cfu/ml, and P4: LAB of 1×10^9 cfu/ml.

greater decrease in blood lipid concentrations. The reduced serum level of cholesterol, triglycerides, HDL, and LDL indicated that the LAB derived from Kumpai Tembaga silage has a hypocholesterolemic effect on Pegagan ducks. Other studies also described the reduced lipid concentration in birds serum due to probiotic supplementation, including LDL, total cholesterol, and triglyceride (Mansoub, 2010; Ashayerizadeh et al., 2011; Shokryazdan et al., 2017). Despite, some studies revealed the opposite results that probiotics did not have a significant effect on the bird's serum total cholesterol (Abdel-Hafeez et al., 2017), HDL (Khan et al., 2013) and LDL (Panda et al., 2006). On the other hand, other studies also reported that probiotics were not able to exert a significant influence on the status of serum protein in poultry. It was confirmed that probiotics were unable to significantly modify the concentration of total protein, albumin, and globulin in chickens (Alkhalf et al., 2010; Abdel-Hafeez et al., 2017; Tayeri et al., 2018). If it was compared, the total concentration of serum protein, albumin, and globulin in this study was higher than the others, namely 4.11-4.19 g/dL, 1.03-1.13 g/dL, and 3.01-3.22 g/dL, respectively. The inconsistent results might be due to differences in probiotic strains, concentrations, or administration procedures. Additionally, differences in serum lipid and protein concentrations in poultry are also determined based on sex. This is as reported by Ghasemi-Sadabadi et al. (2019) that probiotics only had a marked effect on serum cholesterol and total protein in broiler males, while in females are LDL and cholesterol.

It is suggested that the significantly decreased lipid concentration might be associated with degraded cholesterol absorption or synthesis in the gastrointestinal tract with probiotic supplementation. The probiotics could also reduce blood cholesterol by deconjugating bile salts in the intestine duct, which inhibited them from becoming precursor in cholesterol synthesis (Youssef et al., 2017). This is in line with Alkhalf et al. (2010) that *Lactobacillus acidophillus*, one of the LAB strains, had a high bile salt hydrolytic activity that is closely associated

with the deconjugation of bile salts. The deconjugated bile acids have characteristics that are less soluble at low pH. The LAB used in this study is acidophilic, which can produce lactic acid and reduce pH, consequently, those bile acids are less likely absorbed in the small intestine and more eliminated in excreta. Basically, probiotics have some prominent roles in synthesizing bile salt hydrolase (BSH) enzymes, assimilating cholesterol, leading to higher excretion of fecal bile acids, converting cholesterol to coprostanol by cholesterol reductase, and inhibiting the enzyme activity involved in cholesterol synthesis pathway, such as hydroxymethyl-glutaryl-coenzyme A (HMG CoA) reductase (Shokryazdan et al., 2017). Besides, this is also presumably due to the high level of cecal volatile fatty acids (VFAs) which can repress the hepatic cholesterol synthesis (Tang et al., 2017). This is supported by Mookiah et al. (2014) who found that broiler chickens supplemented by probiotics experienced significantly increased caecal VFAs at 21 and 42 d of life. This is also in line with Al-Khalaifa et al. (2019) that caeca provide an anaerobic environment that is suitable for LAB growth and production of undissociated volatile fatty acids (acetic, butyric, propionic, and lactic acids) characterized by acidic pH in caeca.

CONCLUSION

Based on *in vivo* measurements, it can be concluded that the LAB isolated from the Kumpai Tembaga silage is potential enough to be implemented as an alternative additive for Pegagan ducks. The LAB are confirmed able to improve live body weight and increase the length and relative weight of several segments of the small intestine and ceca, which play a significant role in enhancing digestion and nutrient absorption. Additionally, the LAB has been noted to reduce serum lipid concentrations, including cholesterol, triglycerides, LDL, and HDL.

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NOVELTY STATEMENT

Our team has succeeded in discovering and isolating lactic acid bacteria (LAB) derived from Kumpai Tembaga silage, which are potentially used as probiotics. In this study, we conducted *in vivo* observations on Pegagan ducks, which is one of the local ducks from Indonesia. The treatment offered to ducks is the variation of LAB concentration. Our findings recorded that this LAB could improve live body weight, increase the length and relative weight of the small intestine and caeca, and reduce serum cholesterol levels in Pegagan Ducks. The higher concentration of LAB administered tends to provide better results.

AUTHORS CONTRIBUTION

This work was performed in collaboration with all authors. FY, SS, and NG conceptualized the study plan and the design of the experiment. FY, MLS, and ES carried out the fieldwork and collected samples. FY and SS performed the statistical analysis and interpreted the data. FY wrote the draft manuscript. All authors were concerned with revising the manuscript and approved the final revision.

CONFLICT OF INTERESTS

All authors confirm that there is no conflict of interest related to the publication of this paper

ETHICAL APPROVAL

ll procedures are in accordance with the ethical standard of the Sriwijaya University and the regulation of the Republic of Indonesia No. 19 in 2009 regarding animal farming, health and welfare.

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