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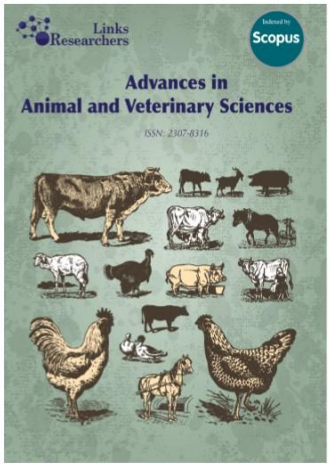

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Probiotics Potential of Lactic Acid Bacteria Derived from Kumpai Tembaga Silage: Effects on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks

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With the consent of all authors and permission from the appropriate authority (*if needed*), I am submitting this manuscript (title is given below) to Advances in Animal and Veterinary Sciences for possible publication in near future. This is the only journal where the work has been submitted and has not been published elsewhere (in part or full).

Title of manuscript: Probiotics potential of lactic acid bacteria derived from Kumpai Tembaga silage: Effects on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks

This study was performed to investigate the influence concentration of lactic acid bacteria (LAB) probiotics 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. Our findings recorded that this LAB probiotic can improve live body weight, increase the length and relative weight of the small intestine and caeca, and reduce serum cholesterol levels in Pegagan Ducks

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RESEARCH ARTICLE

PROBIOTICS POTENTIAL OF LACTIC ACID BACTERIA DERIVED FROM KUMPAI TEMBAGA SILAGE: EFFECTS ON LIVE BODY WEIGHT, GASTROINTESTINAL TRACT, INTERNAL ORGANS, AND BLOOD PROFILES IN PEGAGAN DUCKS

Probiotics Potential of Lactic Acid Bacteria Derived from Kumpai Tembaga Silage: Effects on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks

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Statement of novelty: 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 probiotics concentration. Our findings recorded that this LAB probiotic can 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 probiotics administered tends to provide better results.

Ethical approval (if needed): (All 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)

Probiotics potential of lactic acid bacteria derived from Kumpai Tembaga silage: Effects on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks

ABSTRACT

Probiotics are living microorganisms that provide health benefits to the host by improving the intestine microbial balance. This study was performed to investigate the influence concentration of lactic acid bacteria (LAB) probiotics 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 probiotics), the second to the fifth treatment was LAB probiotics supplementation with a concentration of $1x10^6$, 10^7, 10^8, and 10^9 cfu/ml, respectively. At the 8 weeks of age, sample collection was conducted to determine parameters, including the live body weight, length and relative weight of the gastrointestinal tract and internal organs, and examine hematological and serum biochemical parameters. The administration of LAB probiotics with various concentrations has improved the live body weight and increased the length and relative weight of the total small intestine, duodenum, jejunum, and caeca. Moreover, LAB probiotic 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 probiotics resulted in the greater decrease in serum lipids. It can be concluded that the potential of LAB probiotics 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

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

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 (Pokorná et al., 2019), having cellulolytic activity (Herdian et al., 2018), producing undissociated volatile fatty acids (Al-Khalaifah, 2018), high ability to attach in the intestinal epithelium (Shokryazdan et al., 2017), reducing colonization of pathogenic bacteria (Kim et al., 2015), and resistant to the bile salts influence (Martin et al., 2018). There are several genera of LAB that are widely used as 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, such as increasing the weight gain (Lan et al., 2017), the relative weight of internal organs, and immune response (Al-Khalaifah, 2018). In recent years, studies have been performed by isolating LAB from various sources to be a probiotic candidate for poultry, especially 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, such as colon (Martin et al., 2018), bile (Shi et al., 2020), and caecum (Aziz et al., 2019).

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). The concentration of probiotics is one of the crucial factors to be considered in achieving optimal growth performance. Some studies reported that there are variations regarding the response of poultry to the different probiotic concentrations. A study showed that administering

probiotics 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). However, other studies reported that optimal growth is obtained with the use of probiotics 10^8 cfu (Zhang et al., 2012). Therefore, this in vivo study aims to investigate the influence concentrations of LAB probiotic 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

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 probiotics 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 1-2 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. Probiotics concentration treatments were as follows: P0 (control; without probiotics); P1 (LAB probiotics of 1×10^6 cfu/ml); P2 (LAB probiotics of 1×10^7 cfu/ml), P3 (LAB probiotics of 1×10^8 cfu/ml), and P4 (LAB probiotics of 1×10^9 cfu/ml). Probiotics were offered orally and gradually adjusted to the beak size. In the first 3 weeks of age, ducks were provided 3 ml/bird. Afterward, birds were administrated with probiotics 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 probiotic concentration

In this study, The LAB probiotics 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.

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.

Statistical analysis

Data were analyzed with ANOVA procedure using the SPSS software version 17. The significance of mean differences among treatments was tested by Duncan's multiple range test at 5% of a significance level.

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 probiotics treatments. According to the concentration level of probiotics, a notable effect ($p<0.05$) on body weight occurred when ducks were

147 administered probiotics starting at 107 cfu/ml and above compared to control treatment. The
148 heightened body weight in this study was in line with the other studies (Shokryazdan et al.,
149 2017; Abdel-Hafeez et al., 2017; Park et al., 2018), who stated that supplementation of
150 probiotics was able to increase body weight gain and gain a greater body weight compared to
151 the non-probiotic treatment in the whole experiment. These findings are also in agreement
152 with Mohammadi Gheisar et al. (2016) and Lan et al. (2017) that dietary probiotics
153 containing *Enterococcus faecium* were able to improve the live body weight of chickens
154 compared with the control treatment. The favorable effects of probiotics in increasing body
155 weight indicate that there are an enhanced intestinal digestive enzyme activity and improved
156 nutrients digestion and absorption in the gastrointestinal tract (Mohammadi Gheisar et al.,
157 2016; Park et al., 2016). According to Chen et al. (2013), the activity of digestive enzymes
158 covering protease, amylase, and lipase was enhanced by the role of probiotics, hence
159 optimizing digestion and uptake of nutrients in the gastrointestinal tract. This explanation is
160 also confirmed by Wang and Gu (2010), that bacteria, primarily those belonging to the
161 Bacillus genus, are capable of secreting exoenzymes and might stimulate the production of
162 endogenous enzymes synthesized by the digestive tract of poultry, including amylase,
163 protease, and lipase. In this study, a meaningful increase in live body weight happened when
164 ducks consumed probiotics starting at 10^7 cfu/ml. However, a different result presented by
165 Wang and Gu (2010) that the administration of probiotic *B. coagulans* NJ0516 of 10^6 cfu/g
166 via basal diet was able to significantly increase the final body weight of broilers. With an
167 equal age at 8 weeks, the final live body weight of ducks obtained in this study was slightly
168 lower compared to the body weight reported by Bidura et al. (2019) who was experimenting
169 with the provision of probiotics containing *Saccharomyces spp.* KB-5, *Saccharomyces spp.*
170 KB-8 or the recombination, which was 1.46 – 1.51 kg, whereas in this study the values were

171 ranged from 1.17 to 1.37 kg. In this regard, differences in strains of probiotics have a major
172 effect on the response to body weight gain (Khan et al., 2013).
173
174 **The length and relative weight of the gastrointestinal tract and internal organs**
175 Another significant result ($p<0.05$) was noted in the relative weight and length of
176 gastrointestinal segments, among others total small intestine, duodenum, jejunum, and ceca.
177 While for crop-esophagus, proventriculus, ileum, and colon, it has not presented a notable
178 effect ($p>0.05$) on both weight and length (Table 2). Insignificant results ($p>0.05$) were also
179 recorded in the relative weight of all internal organs, including the gizzard, liver, heart,
180 spleen, pancreas, and bile (Table 3). The increased relative weight of small intestine, jejunum
181 and cecum occurred while ducks were supplemented with probiotics of 10^8 cfu/ml, except for
182 the duodenum which was beginning to increase at 10^6 cfu/ml. While the length of the small
183 intestine and ceca, a significant improvement ($p<0.05$) occurred after providing probiotics of
184 10^6 cfu/ml. It is assumed that probiotics supplementation in this study has been able to
185 enhance the metabolic rate and ultimately increase the relative weight and size of
186 gastrointestinal parts, particularly in the small intestine (Abdel-Hafeez et al., 2017). Many
187 studies associated with the administration of probiotics also documented significant and
188 insignificant results on the weight of the digestive tract and internal organs. Comparable to
189 our findings, Park and Kim (2014) reported that the relative weights of some internal organs
190 were not changed by the administration of LAB probiotics, *B. subtilis* B2A, with
191 concentrations of 10^4-10^6 cfu. This result was also supported by Balamuralikrishnan et al.
192 (2017) that the provision of probiotics, including the Bacillus and Clostridium genus of 10^8
193 and 10^9 cfu/g, did not show a significant impact on the weight of gizzard and other internal
194 organs. In addition, the increased relative length of jejunum was also conferred by Reis et al.
195 (2017) with the supplementation of probiotics of B. subtilis in broiler chicken's diet. The

196 greater relative weight and length of the small intestine and caeca might be influenced by
197 probiotic activity that improves intestinal morphology, including villus height and crypt
198 depth. This is as published by other studies that the administration of probiotics was able to
199 increase the villus height and villus height-to-crypt depth ratio in the small intestine of broiler
200 (Sen et al., 2012; Lei et al., 2015; Agboola et al., 2015). The higher villus height will lead to
201 the enlarged intestinal surface area (Tang et al., 2017), which has the potential to improve the
202 relative weight and length of the small intestine. Furthermore, Hossain et al. (2015) stated
203 that increased villus height and villus height-to-crypt depth ratio are directly related to
204 enhanced epithelial turnover and longer villi were associated with activation of cell mitosis.
205 In contrast to our findings, Abdel-Hafeez et al. (2017) noticed that probiotics did not
206 significantly affect the relative weight of the small intestine (2.61%) in chickens at the end of
207 the finisher period. A reverse result was also reported by Reis et al. (2017) that birds
208 supplemented with LAB strains, *B. subtilis*, definitely presented a reduced relative duodenum
209 length. On the other hand, Aalaei et al. (2018) also reported that none of the jejunal
210 morphological parameters changes in broilers supplemented with probiotics. It can be
211 considered that variations in the strains, sources, viability, and concentrations of bacteria, and
212 methods of administration might be the main factors causing different responses in poultry
213 gastrointestinal tract.

214 **Blood hematological parameters**

215 According to hematological analysis, there were no significant differences ($p>0.05$) between
216 the probiotics supplementation and control groups in all hematological parameters, including
217 Hb, RBC, WBC, thrombocytes, PCV, MCV, MCH, and MCHC, but all of these parameters
218 were within the normal ranges (Table 4). These insignificant results indicate that the
219 concentration of LAB probiotic derived from Kumpai Tembaga silage was not been able to
220 influence blood hematological values. The unmarked hematological parameters in this study

221 are in line with other studies related to probiotic supplementation. The numbers of RBC and
222 WBC in birds was reported not to be significantly increased by the administration of various
223 LAB probiotic strains, such as *Bacillus subtilis* RX7 and B2A (Park and Kim, 2014), *E.*
224 *faecium* (Lan et al., 2017), and *B. subtilis* RX7 and C14 (Park et al., 2018), with the amounts
225 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),
226 and 2.17-2.22 (10^6/μL) and 29.2-31.0 (10^3/μL), respectively. Those RBC and WBC values
227 appear to be lower than that of this study, namely 4.20-4.50 (10^6/μL) and 26.04-29.00
228 (10^3/μL). Additionally, the level of Hb, which is essential in oxygen transport, was also not
229 significantly different between control and probiotics supplementation groups (Alkhalf et al.,
230 2010; Khan et al., 2013). Based on sex differentiation, there was also no notable effect of
231 administrating probiotics to the RBC, WBC, and Hb counts in broiler chicken male and
232 female aged 42 d of life (Ghasemi-Sadabadi et al., 2019). Probiotics might be able to improve
233 the acidic conditions in the digestive tract induced by the fermentation process, which
234 conclusively can absorb more iron for the formation of blood hemoglobin (Abdel-Hafeez et
235 al., 2017). The insignificant influence of probiotics on thrombocyte count and other
236 haemogram parameters, including MCH, MCV, MCHC, and PCV, was also reported by
237 Tang et al. (2017), which was using probiotic PrimaLac on observed laying hens at 36 and 52
238 wk of life. This is also in line with Al-Khalaifa et al. (2019) on 5-wk-broiler chickens
239 supplemented with probiotics of Bacillus and Lactobacillus.
240
241 **Serum biochemical parameters**
242 The administration of LAB probiotics significantly influenced ($p<0.05$) the serum level of
243 cholesterol, triglycerides, HDL, and LDL of Pegagan ducks. However, the level of total
244 protein, albumin, and globulin in serum was not affected ($p>0.05$) by all probiotics
245 concentration treatments (Table 5). Further, ducks fed the higher level of probiotics resulted

246 in a greater decrease in blood lipid concentrations. The reduced serum level of cholesterol,
247 triglycerides, HDL, and LDL indicated that the LAB probiotic derived from Kumpai
248 Tembaga silage has a hypocholesterolemic effect on Pegagan ducks. Other studies also
249 described the reduced lipid concentration in birds serum due to probiotic supplementation,
250 including LDL (Shokryazdan et al., 2017), total cholesterol (Ashayerizadeh et al., 2011), and
251 triglyceride (Mansoub, 2010). Despite, some studies revealed the opposite results that
252 probiotics did not have a significant effect on the bird's serum total cholesterol (Abdel-Hafeez
253 et al., 2017), HDL (Khan et al., 2013) and LDL (Panda et al., 2006). On the other hand, other
254 studies also reported that probiotics were not able to exert a significant influence on the status
255 of serum protein in poultry. It was confirmed that probiotics were unable to significantly
256 modify the concentration of total protein, albumin, and globulin in chickens (Alkhalf et al.,
257 2010; Abdel-Hafeez et al., 2017; Tayeri et al., 2018). If it was compared, the total
258 concentration of serum protein, albumin, and globulin in this study was higher than the
259 others, namely 4.11-4.19 g/dL, 1.03-1.13 g/dL, and 3.01-3.22 g/dL, respectively. The
260 inconsistent results might be due to differences in probiotic strains, probiotic concentrations,
261 or probiotic administration procedures. Additionally, differences in serum lipid and protein
262 concentrations in poultry are also determined based on sex. This is as reported by Ghasemi-
263 Sadabadi et al. (2019) that probiotics only had a marked effect on serum cholesterol and total
264 protein in broiler males, while in females are LDL and cholesterol.

265 It is suggested that the significantly decreased lipid concentration might be associated with
266 degraded cholesterol absorption or synthesis in the gastrointestinal tract with probiotic
267 supplementation. The probiotics could also reduce blood cholesterol by deconjugating bile
268 salts in the intestine duct, which inhibited them from becoming precursor in cholesterol
269 synthesis (Youssef et al., 2017). This is in line with Alkhalf et al. (2010) that *Lactobacillus*
270 *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 probiotic 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. In principle, probiotics have some prominent roles in the hypocholesterolemia mechanism, consisted of 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, namely 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 probiotic LAB isolated from the Kumpai Tembaga silage is potential enough to be implemented as an alternative additive for Pegagan ducks. LAB probiotics 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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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

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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
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).

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

Traits	Concentration of probiotics				
	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 probiotics, P1= LAB probiotics of 1×10^6 cfu/ml, P2= LAB probiotic of 1×10^7 cfu/ml, P3= LAB probiotics of 1×10^8 cfu/ml, and P4= LAB probiotics of 1×10^9 cfu/ml.

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

Traits	Concentration of probiotics				
	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 probiotics, P1= LAB probiotics of 1×10^6 cfu/ml, P2= LAB probiotic of 1×10^7 cfu/ml, P3= LAB probiotics of 1×10^8 cfu/ml, and P4= LAB probiotics of 1×10^9 cfu/ml.

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

Traits	Concentration of probiotics				
	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 probiotics, P1= LAB probiotics of 1×10^6 cfu/ml, P2= LAB probiotic of 1×10^7 cfu/ml, P3= LAB probiotics of 1×10^8 cfu/ml, and P4= LAB probiotics of 1×10^9 cfu/ml.

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

Traits	Concentration of probiotics				
	P0	P1	P2	P3	P4
Cholesterol (mg)	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)	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)	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)	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 ± 0.42	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 probiotics, P1= LAB probiotics of 1×10^{6} cfu/ml, P2= LAB probiotic of 1×10^{7} cfu/ml, P3= LAB probiotics of 1×10^{8} cfu/ml, and P4= LAB probiotics of 1×10^{9} cfu/ml.

2. Bukti konfirmasi manuskrip telah di-*assigned* ke Editor Tahap 1 (21 Mei 2020)

Fitra Yosi unsri <fitrayosi@unsri.ac.id>

Your manuscript in Advances in Animal and Veterinary Sciences has been assigned an Editor

1 pesan

Manuscript Handler <info@manuscripthandler.com> 21 Mei 2020 pukul 02.29
Balas Ke: Manuscript Handler <info@manuscripthandler.com>, Nexus Academic Publishers <info@nexusacademicpublishers.com>
Kepada: fitrayosi@unsri.ac.id

Dear Mr. Fitra Yosi,

Your manuscript entitled Probiotics Potential of Lactic Acid Bacteria Derived from Kumpai Tembaga Silage: Effects on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks has passed initial quality controls and is now been assigned an Editor. After editorial considerations, the manuscript will be sent to selected reviewers for peer-review process. Please note that review process is on the disposal of reviewer's responses. We strive our best to make first decision at the earliest possible; however, your patience in this matter will be highly appreciated.

You can also view the status of your manuscript at any time by checking your Author Center after logging in to
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Thank you for submitting your manuscript and we will keep you updated with any further progress in the peer-review process of the manuscript.

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3. Bukti konfirmasi manuskrip telah di-*assigned* ke Reviewer Tahap 1 (25 Mei 2020)

Fitra Yosi unsri <fitrayosi@unsri.ac.id>

Your Manuscript in Advances in Animal and Veterinary Sciences has been assigned Reviewers

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Dear Mr Fitra Yosi,

Your Manuscript ID MH20200520100506 with title Probiotics Potential of Lactic Acid Bacteria Derived from Kumpai Tembaga Silage: Effects on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks has been assigned reviewers. We will try our best to have reviewer's feedback at their earliest possible and to reduce the time from submission to publication. However, please note that some reviewers take longer time than anticipated which overall effect the peer-review time. We would appreciate your patience in this matter.

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4. Bukti konfirmasi *Editor's decision* terhadap hasil review Tahap 1, serta *list* komentar Editor dan Reviewer (12 Juni 2020)

Fitra Yosi unsri <fitrayosi@unsri.ac.id>

Your Manuscript in Advances in Animal and Veterinary Sciences is awaiting Editor's decision

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Balas Ke: Manuscript Handler <info@manuscripthandler.com>, Nexus Academic Publishers <info@nexusacademicpublishers.com>
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Dear Mr Fitra Yosi,

We have received reviewer's reports or editor's assessments for your Manuscript ID MH20200520100506 with title Probiotics Potential of Lactic Acid Bacteria Derived from Kumpai Tembaga Silage: Effects on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks in Advances in Animal and Veterinary Sciences.

The manuscript is with Editor to make final decision.

This decision may take some time as it is being discussed with the Editorial Board members. Not hearing the decision on your manuscirpt indicates that the decision is not yet agreed. As soon as the decision is committed, you will be informed.

This email is for your information only and there is nothing for you to do at this moment. We will keep you updated with further information.

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Nexus Academic Publishers: Decision on Manuscript ID MH20200520100506

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Manuscript Handler <info@manuscripthandler.com> 12 Juni 2020 pukul 18.35
Balas Ke: Manuscript Handler <info@manuscripthandler.com>, Nexus Academic Publishers <mohammedvet1986@gmail.com>
Kepada: fitrayosi@unsri.ac.id
Cc: nexusacademicsonline@gmail.com

Fri, 12 Jun 2020, 12:34 PM

Dear Mr. Fitra Yosi,

We have received the reports from our reviewers on your manuscript, "Probiotics Potential of Lactic Acid Bacteria Derived from Kumpai Tembaga Silage: Effects on Live Body Weight, Gastrointestinal Tract, Internal Organs, and Blood Profiles in Pegagan Ducks", which you submitted to Advances in Animal and Veterinary Sciences with MH20200520100506.

Based on the received comments, your manuscript could be reconsidered for publication, should you be prepared to incorporate Major Revisions.

The comments and requests of the Editor and the Peer Reviewers are included below. Please share this information with all coauthors of the manuscript.

Editor's Comments:

• Review the peer review comments and requests carefully, and edit the manuscript accordingly.

• Include a separate point-by-point response file addressing the reviewers comments along with an explanation of any request of the editor or the reviewers that you do not address in your revised manuscript. Your list of responses should be uploaded as a Cover Letter in addition to your revised manuscript.

• Please colour (e.g. red in contrast to black text) all changes in the revised manuscript, without such coloured changes the manuscript may be returned or rejected.

• Verify the placement and accuracy of each reference in your manuscript as well as the accuracy of all of the values in your tables and figures.

• Please ensure that all author's names and their affiliations are placed correctly.

• Make every effort to address the remaining concerns and to resubmit your manuscript. If you anticipate an additional delay, or if you do not wish to resubmit your manuscript, then please notify us as soon as possible.

• Please keep your coauthors apprised of the status of the article throughout the revision process.

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Username: fitrayosi@unsri.ac.id
Password: fitra0019068502

We look forward to receiving your revised manuscript.

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Reviewer(s) Comments to Author:

Reviewer: 1:

Comments to the Author

1- use SAS for statistical analysis
2- determine type of analysis (One way or two way)
3- determine the type of statistical design

Reviewer: 2:

Comments to the Author

This experiment is interesting because of research innovation. As we know, most of LAB have been used as the probiotic. However, before using as the probiotic, many experiments and confirmation are needed to meet many criteria of a probiotic, eg. safety criteria, technological criteria, functional criteria and desirable physiological criteria. After confirming some of those criteria, the product (LAB) could be say as "Probiotic". In this experiment, the author used the LAB isolated from the ensiled material. The research idea, use of LAB as direct fed microorganism (DFM) as feed additive, is very good. But, it is too early to nominate this LAB as "Probiotic" without confirmation for probiotic criteria. So, I would like to suggest to the authors that it would be used as "Supplementation of LAB derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks" instead of using "Probiotics potential of lactic acid bacteria derived from Kumpai Tembaga silage: Effects on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks".

Reviewer: 3:

Comments to the Author

Comments to the Author
General comments
This manuscript tested Probiotics potential of lactic acid bacteria derived from Kumpai Tembaga silage on duck. This trial is relevant and presents significant contributions to poultry production and environmental impact. And also, the results from this study could be helpful to the duck producers. Material and Methods well organized, and results and discussion also explained clearly. However, it still needed to be improved in many parts for the publication.

Specific comments
P3 L58-60: Please change this text.

P3 L61: Delete ‘has’.

P4 L85-86: Insert ‘(Park and Kim, 2014),’.

P4 L88-90: Change ‘are well documented (Park and Kim, 2014Balamuralikrishnan et al., 2017; Upadhaya et al., 2019)’.

P4-5 L92-96: Please change this text shortly like P4 L88-90: Change ‘ are well documented (Park and Kim, 2014Balamuralikrishnan et al., 2017; Upadhaya et al., 2019)’.

P5 L100-101: Change ‘in poultry (Lan et al., 2017; Al-Khalaifah, 2018)’.

P5 L107-108: Change ‘in poultry (Aziz et al., 2019; Martin et al., 2018; Shi et al., 2020)’.

P5-6 L116-119. This sentence needed to be clarified more clearly.

P6 L126: Need ‘Ethics statement’.

P6 L132: Change ‘from 0-2 and 3-8 weeks of life’.

P6 L139: What is 3 ml/bird?

P8 L187: You should follow the journal guidelines to separate results and discussion section.

P9 L191: Change ‘at 107cfu/ml’.

P9 L206: Change ‘of poultry (amylase, protease, and lipase)’.

P10 L221-228: This sentence needed to be clarified more clearly or moved other lines to make clear.

P11 L241: Delete ‘including’.

P11 L242-243: Please change this text.

P11 L258: What are the reasons on no all hematological paramenters when probiotics were used in this study?
P11 L260-261: Delete ‘including Hb, RBC, WBC, thrombocytes, PCV, MCV, MCH, and MCHC,’.

P13 L293-297: Please change this text.

P14 L319-323: Please change this text.

Download additional comments

Reviewer: 4:

Comments to the Author

1. Introduction is too long
2. Materials and methods should provide enough information, such test kit use, measurement,
3. Please explain from the your study. why it is different from other studies?
4. Double check the citation in the text and reference sections.
5. Double check Scientific name throughout the manuscript
Why dried salt is not included in the experimental diets?
Why the amount of premix is not met the NRC recommendation?

Download additional comments

5. Bukti konfirmasi re-submit revisi manuskrip, respon kepada reviewer/editor, dan artikel yang direvisi/diresubmit (28 Juni 2020)

Fitra Yosi unsri <fitrayosi@unsri.ac.id>

Manuscript MH20200520100506-R1 is submitted to Journal Advances in Animal and Veterinary Sciences

1 pesan

Manuscript Handler <info@manuscripthandler.com> 28 Juni 2020 pukul 01.26
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Dear Fitra Yosi,

Your manuscript entitled "Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks" has been successfully submitted online and is presently being given full consideration for publication in the Advances in Animal and Veterinary Sciences.

Your manuscript ID is MH20200520100506-R1

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in at http://manuscripthandler.com/nexus/Advances-in-Animal-and-Veterinary-Sciences and edit your user information as appropriate.

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Thank you for submitting your manuscript to the Advances in Animal and Veterinary Sciences.

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Responds to Editor's Comments:

1. Review the peer review comments and requests carefully, and edit the manuscript accordingly. (has been reviewed in the revised manuscript)
2. Include a separate point-by-point response file addressing the reviewers comments along with an explanation of any request of the editor or the reviewers that you do not address in your revised manuscript. Your list of responses should be uploaded as a Cover Letter in addition to your revised manuscript. (has been included in Cover Letter)
3. Please colour (e.g. red in contrast to black text) all changes in the revised manuscript, without such coloured changes the manuscript may be returned or rejected.(changes in revised manuscript has been coloured in red)
4. Verify the placement and accuracy of each reference in your manuscript as well as the accuracy of all of the values in your tables and figures.(has been verified in the revised manuscript)
5. Please ensure that all author's names and their affiliations are placed correctly. (has been ensured)
6. Make every effort to address the remaining concerns and to resubmit your manuscript. If you anticipate an additional delay, or if you do not wish to resubmit your manuscript, then please notify us as soon as possible.(has been done)
7. Please keep your coauthors apprised of the status of the article throughout the revision process. (has been done)

Responds to the comments of Reviewer 1:

1- use SAS for statistical analysis (Basically, between SAS and SPSS software have in common in processing data. In this case, we chosed SPSS)
2- determine type of analysis (One way or two way) (has beed added in the body text, page 6 line 205-207)
3- determine the type of statistical design (has beed added in the body text, page 6 line 205-207)

Responds to the comments of Reviewer 2:

I would like to suggest to the authors that it would be used as "Supplementation of LAB derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks" instead of using "Probiotics potential of lactic acid bacteria derived from Kumpai Tembaga silage: Effects on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks".
(The title of manuscript has been changed in the revised manuscript, Page 1, line 1-2)

Responds to the comments of Reviewer 3:

P3 L58-60: Please change this text. (has been changed in abstract, line 13-15)
P3 L61: Delete 'has'. (has been deleted in abstract, line 15)
P4 L85-86: Insert '(Park and Kim, 2014),'. (has been inserted in introduction, line 61)

P4 L88-90: Change 'are well documented (Park and Kim, 2014; Balamuralikrishnan et al., 2017; Upadhaya et al., 2019)'.(has been changed in introduction, line 62-65)
P4-5 L92-96: Please change this text shortly like P4 L88-90: Change ' are well documented (Park and Kim, 2014; Balamuralikrishnan et al., 2017; Upadhaya et al., 2019)'. (has been changed in introducton, line 68-84)
P5 L100-101: Change 'in poultry (Lan et al., 2017; Al-Khalaifah, 2018)'. (has been changed in introduction, line 88-90)
P5 L107-108: Change 'in poultry (Aziz et al., 2019; Martin et al., 2018; Shi et al., 2020)'.(has been changed in introduction, line 94-95)
P5-6 L116-119. This sentence needed to be clarified more clearly. (has been clarified in introduction, line 101-107)
P6 L126: Need 'Ethics statement'. (ethics statement has been inserted, line 134-136)
P6 L132: Change 'from 0-2 and 3-8 weeks of life'. (has been changed, line 142)
P6 L139: What is 3 ml/bird? (has been clarified, line 148)
P8 L187: You should follow the journal guidelines to separate results and discussion section. (the jurnal guidelines has been followed)
P9 L191: Change 'at 10^7cfu/ml'. (has been changed, line 214)
P9 L206: Change 'of poultry (amylase, protease, and lipase)'. (has been changed, line 237)
P10 L221-228: This sentence needed to be clarified more clearly or moved other lines to make clear. (the sentence has been clarified, line 251-261)
P11 L241: Delete 'including'. (has been deleted, line 277)
P11 L242-243: Please change this text. (the text has been changed, line 277-278)
P11 L258: What are the reasons on no all hematological paramenters when probiotics were used in this study? (the reason has been explained, line 303-304 and line 320-322)
P11 L260-261: Delete 'including Hb, RBC, WBC, thrombocytes, PCV, MCV, MCH, and MCHC,'. (has been revised, line 301-302)
P13 L293-297: Please change this text. (the text has been change, line 337-338)
P14 L319-323: Please change this text. (the text has been changed, line 368-380)

Responds to the comments of Reviewer 4:

1. Introduction is too long (introduction has been revised, line 50-131)
2. Materials and methods should provide enough information, such test kit use, measurement (Information about material and methods has been explained, line 133-202)
3. Please explain from the your study. why it is different from other studies?(the difference between this study and others studies has been explained in introduction, line 96-101)
4. Double check the citation in the text and reference sections. (has been double-checked)
5. Double check Scientific name throughout the manuscript (has been double-checked)
6. Why dried salt is not included in the experimental diets?(because it has been fulfilled by other feed ingredients)
7. Why the amount of premix is not met the NRC recommendation? (the amount of vitamins, minerals, and amino acids contained in the premix has been fulfilled for ducks)

Additional comments from Reviewer 4 in the manuscript:

Comment [W1]: Lactobacillus, Enterococcus, and Bifidobacterium in italic (have been corrected, line 86-87)
Comment [W2]: Is very strength. Double check (has been double checked)
Comment [W3]: Please specify the methods for analyzing of all blood profiles. It is not not clear from the citation (the methods has been inserted, line 203).
Comment [W4&W5]: Gheisar? Please correct both in the text and citation (has been matched both in the text and citation)
Comment [W6]: B. Subtilis in italic (has been corrected, line 277)
Comment [W7]: Citation is needed (Ghasemi-Sadabadi et al. (2019) has been added in references, line 471-475)
Comment [W8&W9]: Al-Khalaifah HS (2018) is not found in the text (it is found in the introduction, line 70 and 89)
Comment [W10]: Anas moschata in italic (has been corrected, line 480)
Comment [W11]: Enterococcus faecium in italic (has been corrected, line 528)
Comment [W11]: Change Broiler to broiler (has been changed, line 582)

RESEARCH ARTICLE

SUPPLEMENTATION OF LACTIC ACID BACTERIA DERIVED FROM ENSILED KUMPAI TEMBAGA ON LIVE BODY WEIGHT, GASTROINTESTINAL TRACT, INTERNAL ORGANS, AND BLOOD PROFILES IN PEGAGAN DUCKS

Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks

Fitra Yosi*[1], Sofia Sandi[1], Nuni Gofar[2], Meisji Liana Sari[1], and Eli Sahara[1]

[1)]Department of Animal Science, Faculty of Agriculture, University of Sriwijaya. Jl. Raya Palembang-Prabumulih KM.32, Indralaya, South Sumatra, Indonesia, 30662.
[2)]Department of Soil Science, Faculty of Agriculture, University of Sriwijaya. Jl. Raya Palembang-Prabumulih KM.32, Indralaya, South Sumatra, Indonesia, 30662.

*Corresponding author:
Fitra Yosi,
Department of Animal Science, Faculty of Agriculture, University of Sriwijaya. Jl. Raya Palembang-Prabumulih KM.32, Indralaya, South Sumatra, Indonesia, 30662. e-mail: fitrayosi@unsri.ac.id , phone, fax = +62711 580059, +62711580276

Numbers of tables in the manuscript: 5

Statement of novelty: 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.

Ethical approval (if needed): (All 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)

~~**Probiotics potential of lactic acid bacteria derived from Kumpai Tembaga silage: Effects on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks**~~

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 ~~Probiotics are living microorganisms~~ that provide health benefits to the host by improving the intestine microbial balance. This study was performed to investigate the influence concentration of ~~lactic acid bacteria (~~LAB~~)~~ ~~probiotics~~ 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 ~~probiotics~~), the second to the fifth treatment was LAB ~~probiotics~~ supplementation with a concentration of $1x10^6$, 10^7, 10^8, and 10^9 cfu/ml, respectively. ~~At the 8 weeks of age,~~ ~~S~~samples ~~collection~~ ~~were~~as collected at 8 weeks of life ~~conducted~~ to determine the ~~parameters, including the~~ live body weight, length and relative weight of the gastrointestinal tract and internal organs, and ~~examine~~ hematological and serum biochemical parameters. The administration of LAB ~~probiotics~~ with various concentrations ~~has~~ improved the live body weight and increased the length and relative weight of the total small intestine, duodenum, jejunum, and caeca. Moreover, LAB ~~probiotic~~ 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 ~~probiotics~~ LAB resulted in the greater decrease in serum lipids. It can be concluded that the potential of LAB ~~probiotics~~

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

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 ~~activities~~ (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, ~~by administering probiotics~~ are also well documented by many studies~~, such as increasing body weight gain~~ (Park and Kim, 2014; Balamuralikrishnan

et al., 2017;), improving egg production (Upadhaya et al., 2019), and elevating the relative weight of internal organs (Park and Kim, 2014).

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 (Pokorná et al., 2019), having cellulolytic activity (Herdian et al., 2018), producing undissociated volatile fatty acids (Al Khalaifah, 2018), high ability to attach in the intestinal epithelium (Shokryazdan et al., 2017), reducing colonization of pathogenic bacteria (Kim et al., 2015), 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 as 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 (Lan et al., 2017), the relative weight of internal organs, and immune response (Al Khalaifah, 2018). In recent years, studies have been performed by isolating LAB from various sources to be a probiotic candidate for poultry, especially 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 (Martin et al., 2018), bile (Shi et al., 2020), and caecum (Aziz et al., 2019),.

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

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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 t~~T~~he concentration ~~of probiotics~~ and the strains of bacteria are~~is one of~~ the crucial factors to be considered in achieving optimal growth performance. ~~Some studies reported that there are variations regarding the response of poultry to the different probiotic concentrations~~. A study showed that administering ~~probiotics~~ *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). ~~However~~Meanwhile, another study~~ies~~ reported that optimal growth was obtained with the use of *Bacillus subtilis* of ~~probiotics~~ 10^8 cfu (Zhang et al., 2012). Therefore, this in vivo study aims to investigate the influence concentrations of LAB ~~probiotic~~ 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.

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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 ~~probiotics~~ 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~~1~~-2 and 2-8 weeks of life, respectively (Table 1). Diets were formulated

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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~~Probiotics~~ concentration treatments were as follows: P0 (control; without ~~probiotics~~LAB); P1 (LAB ~~probiotics~~ of 1×10^{6} cfu/ml); P2 (LAB ~~probiotics~~ of 1×10^{7} cfu/ml), P3 (LAB ~~probiotics~~ of 1×10^{8} cfu/ml), and P4 (LAB ~~probiotics~~ of 1×10^{9} cfu/ml). The ~~Probiotics~~ LAB w~~as~~ere 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 ~~probiotics~~ 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 ~~probiotic~~ LAB concentration

In this study, The LAB ~~probiotics~~ 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

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by comparing the diluted LAB solution and the McFarland standard solution based on the level of turbidity.

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 ~~Data were analyzed with ANOVA procedure using the SPSS software version 17. The significance of mean differences among treatments was tested by Duncan's multiple range test at 5% of a significance level.~~

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 ~~probiotics~~ LAB treatments. According to the concentration level of ~~probiotics~~LAB, a notable effect ($p<0.05$) on body weight occurred when ducks were administered ~~probiotics~~ 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 ~~probiotics~~ 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

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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~~, including~~ (amylase, protease, and lipase). In this study, a meaningful increase in live body weight happened when ducks consumed LAB~~probiotics~~ 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 ~~has not~~ presented a~~n~~ unmarked~~notable~~ effect ($p>0.05$) on both weight and length (Table 2). Insignificant results ($p>0.05$) were also recorded in the relative weight of ~~all internal organs, including the~~ gizzard, liver, heart, spleen, pancreas, and bile (Table 3). The increased relative weight of small intestine, jejunum and cecum occurred when~~ile~~ ducks were supplemented with

~~probiotics~~ 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 ~~probiotics~~ 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, ~~including~~ such as villus height and crypt depth. This is also confirmed~~as published~~ 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

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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 ~~probiotics~~ LAB supplementation and control groups in ~~all hematological parameters, including~~ 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 ~~probiotic~~ 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

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*.

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Serum biochemical parameters

The administration of LAB ~~probiotics~~ 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 ~~all probiotics~~LAB concentration treatments (Table 5). Further, ducks fed the higher level of ~~probiotics~~ LAB resulted in a greater decrease in blood lipid concentrations. The reduced serum level of cholesterol, triglycerides, HDL, and LDL indicated that the LAB ~~probiotic~~ 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 ~~(Shokryazdan et al., 2017)~~, total cholesterol ~~(Ashayerizadeh et al., 2011)~~, 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, ~~probiotic~~ concentrations, or ~~probiotic~~ 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 ~~probiotic~~ 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. ~~In principle~~Basically, probiotics have some prominent roles in ~~the hypocholesterolemia mechanism, consisted of~~ 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~~namely~~ 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 ~~probiotic~~ LAB isolated from the Kumpai Tembaga silage is potential enough to be implemented as an alternative additive for Pegagan ducks. The LAB ~~probiotics~~ 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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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

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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
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).

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

Traits	Concentration of probiotics				
	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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^6 cfu/ml, P2= LAB ~~probiotic~~ of 1×10^7 cfu/ml, P3= LAB ~~probiotics~~ of 1×10^8 cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^9 cfu/ml.

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

Traits	Concentration of probiotics				
	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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^6 cfu/ml, P2= LAB ~~probiotic~~ of 1×10^7 cfu/ml, P3= LAB ~~probiotics~~ of 1×10^8 cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^9 cfu/ml.

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

Traits	Concentration of probiotics				
	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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^6 cfu/ml, P2= LAB ~~probiotic~~ of 1×10^7 cfu/ml, P3= LAB ~~probiotics~~ of 1×10^8 cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^9 cfu/ml.

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

Traits	Concentration of probiotics				
	P0	P1	P2	P3	P4
Cholesterol (mg)	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)	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)	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)	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 ± 0.42	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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^{6} cfu/ml, P2= LAB ~~probiotic~~ of 1×10^{7} cfu/ml, P3= LAB ~~probiotics~~ of 1×10^{8} cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^{9} cfu/ml.

6. Bukti konfirmasi manuskrip revisi telah di-*assigned* ke Editor Tahap 2 (29 Juni 2020)

Fitra Yosi unsri <fitrayosi@unsri.ac.id>

Your Manuscript ID: MH20200520100506-R1 has been assigned an Editor

1 pesan

Manuscript Handler <info@manuscripthandler.com> 29 Juni 2020 pukul 16.55
Balas Ke: Manuscript Handler <info@manuscripthandler.com>, Nexus Academic Publishers <info@nexusacademicpublishers.com>
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Dear Mr Fitra Yosi,

Your Manuscript ID MH20200520100506-R1 with title "Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks" has passed the initially quality checks and is now transferred to the Editor of the journal to be considered for review process.

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7. Bukti konfirmasi manuskrip revisi telah di-*assigned* ke Reviewer Tahap 2 (29 Juni 2020)

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Your Manuscript in Advances in Animal and Veterinary Sciences has been assigned Reviewers

Manuscript Handler <info@manuscripthandler.com> 30 Juni 2020 pukul 08.55
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Your Manuscript ID MH20200520100506-R1 with title Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks has been assigned reviewers. We will try our best to have reviewer's feedback at their earliest possible and to reduce the time from submission to publication. However, please note that some reviewers take longer time than anticipated which overall effect the peer-review time. We would appreciate your patience in this matter.

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8. Bukti konfirmasi *Editor's decision* terhadap hasil review Tahap 2, serta *list* komentar Editor dan Reviewer (30 Juni 2020)

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Kepada: fitrayosi@unsri.ac.id

Dear Mr Fitra Yosi,

We have received reviewer's reports or editor's assessments for your Manuscript ID MH20200520100506-R1 with title Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks in Advances in Animal and Veterinary Sciences.

The manuscript is with Editor to make final decision.

This decision may take some time as it is being discussed with the Editorial Board members. Not hearing the decision on your manuscirpt indicates that the decision is not yet agreed. As soon as the decision is committed, you will be informed.

This email is for your information only and there is nothing for you to do at this moment. We will keep you updated with further information.

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Nexus Academic Publishers: Decision on Manuscript ID MH20200520100506-R1

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Tue, 30 Jun 2020, 12:59 PM

Dear Mr. Fitra Yosi,

We have received the reports from our reviewers on your manuscript, "Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks", which you submitted to Advances in Animal and Veterinary Sciences with MH20200520100506-R1.

Based on the received comments, your manuscript could be reconsidered for publication, should you be prepared to incorporate Minor Revisions.

The comments and requests of the Editor and the Peer Reviewers are included below. Please share this information with all coauthors of the manuscript.

Editor's Comments:

• Review the peer review comments and requests carefully, and edit the manuscript accordingly.

• Include a separate point-by-point response file addressing the reviewers comments along with an explanation of any request of the editor or the reviewers that you do not address in your revised manuscript. Your list of responses should be uploaded as a Cover Letter in addition to your revised manuscript.

• Please colour (e.g. red in contrast to black text) all changes in the revised manuscript, without such coloured changes the manuscript may be returned or rejected.

• Verify the placement and accuracy of each reference in your manuscript as well as the accuracy of all of the values in your tables and figures.

• Please ensure that all author’s names and their affiliations are placed correctly.

• Make every effort to address the remaining concerns and to resubmit your manuscript. If you anticipate an additional delay, or if you do not wish to resubmit your manuscript, then please notify us as soon as possible.

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Reviewer(s) Comments to Author:

Reviewer: 1:

Comments to the Author

Please double check units for all measurements of metabolic profile

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9. Bukti konfirmasi re-submit revisi manuskrip, respons kepada reviewer/editor, dan artikel yang direvisi/diresubmit (1 Juli 2020)

Fitra Yosi unsri <fitrayosi@unsri.ac.id>

Manuscript MH20200520100506-R2 is submitted to Journal Advances in Animal and Veterinary Sciences

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Dear Fitra Yosi,

Your manuscript entitled "Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks" has been successfully submitted online and is presently being given full consideration for publication in the Advances in Animal and Veterinary Sciences.

Your manuscript ID is MH20200520100506-R2

Please mention the above manuscript ID in all future correspondence or when calling the office for questions. If there are any changes in your street address or e-mail address, please log in at http://manuscripthandler.com/nexus/Advances-in-Animal-and-Veterinary-Sciences and edit your user information as appropriate.

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Editor-in-Chief,
Advances in Animal and Veterinary Sciences

With the consent of all authors and permission from the appropriate authority (*if needed*), I am submitting this **revised manuscript** (title is given below) to Advances in Animal and Veterinary Sciences for possible publication in near future. This is the only journal where the work has been submitted and has not been published elsewhere (in part or full).

Title of manuscript: Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks

This study was performed to investigate the influence concentration of lactic acid bacteria (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. 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

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Responds to Editor's Comments:

1. Review the peer review comments and requests carefully, and edit the manuscript accordingly. (has been reviewed in the revised manuscript)
2. Include a separate point-by-point response file addressing the reviewers comments along with an explanation of any request of the editor or the reviewers that you do not address in your revised manuscript. Your list of responses should be uploaded as a Cover Letter in addition to your revised manuscript. (has been included in Cover Letter)
3. Please colour (e.g. red in contrast to black text) all changes in the revised manuscript, without such coloured changes the manuscript may be returned or rejected.(changes in revised manuscript has been coloured in red)
4. Verify the placement and accuracy of each reference in your manuscript as well as the accuracy of all of the values in your tables and figures.(has been verified in the revised manuscript)
5. Please ensure that all author's names and their affiliations are placed correctly. (has been ensured)
6. Make every effort to address the remaining concerns and to resubmit your manuscript. If you anticipate an additional delay, or if you do not wish to resubmit your manuscript, then please notify us as soon as possible.(has been done)
7. Please keep your coauthors apprised of the status of the article throughout the revision process. (has been done)

Responds to the comments of Reviewer:

Comment [W1-W5]: Use capital letter (has been changed, in revised tables)
Comment [W6]: Please clarify Total P/available P (has been clarified, available P)
Comment [W7]: Why Met and Lys are include in Premix (Premix used is a commercial premix,which contains methionine and lysine in it)
Comment [W9]: Its better to report as relative length of these measurements (We prefer to display the actual length)
Comment [W11-W19]: Double check unit for all measurement in Table 5 (all units have been dobule checked, in revised tables)

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

Ingredients	Ceomposition (%)	
	starter (0-2 wk)	finisher (2-8 wk)
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Soybean meal	28	16
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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).

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

Traits	Concentration of ~~probiotics~~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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^{6} cfu/ml, P2= LAB ~~probiotic~~ of 1×10^{7} cfu/ml, P3= LAB ~~probiotics~~ of 1×10^{8} cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^{9} cfu/ml.

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

Traits	Concentration of ~~probiotics~~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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^{6} cfu/ml, P2= LAB ~~probiotic~~ of 1×10^{7} cfu/ml, P3= LAB ~~probiotics~~ of 1×10^{8} cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^{9} cfu/ml.

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

Traits	Concentration of ~~probiotics~~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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^6 cfu/ml, P2= LAB ~~probiotic~~ of 1×10^7 cfu/ml, P3= LAB ~~probiotics~~ of 1×10^8 cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^9 cfu/ml.

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

Traits	Concentration of ~~probiotics~~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 ± 0.42	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 ~~probiotics~~, P1= LAB ~~probiotics~~ of 1×10^{6} cfu/ml, P2= LAB ~~probiotic~~ of 1×10^{7} cfu/ml, P3= LAB ~~probiotics~~ of 1×10^{8} cfu/ml, and P4= LAB ~~probiotics~~ of 1×10^{9} cfu/ml.

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Wed, 08 Jul 2020, 12:45 PM

Dear Mr. Fitra Yosi,

It is a pleasure to accept your manuscript entitled "Supplementation of lactic acid bacteria derived from ensiled Kumpai Tembaga on live body weight, gastrointestinal tract, internal organs, and blood profiles in Pegagan ducks" in its current form for publication in the Advances in Animal and Veterinary Sciences.

Your article is now being processed for formatting, copy editing and final publication. You will be informed for each step and we will contact you when we need any further information or material.

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Submitted By:

Mr Fitra Yosi, Dr Sofia Sandi, Dr Nuni Gofar, Dr Meisji L Sari, Dr Eli Sahara,

has been accepted in Advances in Animal and Veterinary Sciences on 08-07-2020

From the Editor

Advances in Animal and Veterinary Sciences

Thanks

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Research Article

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

Fitra Yosi[1]*, Sofia Sandi[1], Nuni Gofar[2], Meisji Liana Sari[1], Eli Sahara[1]

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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 as 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 treatments. 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 both 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 ± 0.42	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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Research Article

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 $1x10^8$, 10^7, 10^6, and 10^5 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 10^5 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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