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The Assays of Bacteria-Yeast Consortia as Probiotics Candidates and Their Influences on Nutrients Utilization of Quails Diet

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ABSTRACT

This study aimed to assay *L. plantarum* (P1), *S. cerevisiae* (P2), and its combination (P3) as probiotics candidates and their effects on nutrient digestibility in Japanese quails (*Cortunix cortunix japonica*) diets. *In vitro* assays were employed to evaluate the antibacterial activities against pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Salmonella pullorum*), sensitivity to antibiotics (i.e., streptomycin, penicillin, and erythromycin), stress on bile salt and acidity condition. Evaluation of probiotics on *in vivo* nutrient utilization was carried out by administration of probiotics to laying quails in drinking water with various treatments (R0, control; R1, *L. plantarum*; R2, *S. cerevisiae*; R3, combination of R1+R2; R4, commercial feed additive). Results showed that the highest inhibitory activity was performed by P2 on *S. aureus* (17.28 mm). Treatments P2 and P3 were resistant to all the tested antibiotics, while P1 was resistant to streptomycin and penicillin. P1 was tolerant to bile salts whereas P2 was tolerant to gastric acidity conditions. For the *in vivo* experiment, quails fed R1, R2, and R4 had higher nitrogen retention as compared to R0 ($p<0.05$). However, all treatments revealed similar metabolizable energy values. The cluster analysis showed that quails consuming the combination of *L. plantarum* and *S. cerevisiae* were within the same cluster with the control but different clusters from the individual probiotics across all parameters, indicating a possible antagonistic effect between the two species. In conclusion, the inclusion of *L. plantarum* or *S. cerevisiae* inhibits pathogenic bacteria without influencing nutrient utilization of quail diet.

***Keywords:** antibiotic growth promoters; inhibitory activity; lactic acid bacteria; microbial feed additive; quails*

INTRODUCTION

Antibiotic growth promoters (AGPs) is commonly used as feed additive to enhance poultry performance. However, dietary inclusion of AGPs had a negative effect on poultry such as the resistance of pathogenic bacteria in gastrointestinal tract (GIT) and antibiotic residue in poultry products (Huyghebaert *et al*., 2011). In 2006, AGPs was banned in the European Union countries (Ronquillo & Hernandez, 2017), followed by some ASEAN countries (Goutard *et al*., 2017) including Indonesia has also banned AGPs use for animal feed in early 2018 by implementation of the animal medicine classification act (MARI, 2017).

Exploration of probiotics candidate as an alternative feed additive for replacing AGPs had been conducted by many researchers in the current decade. Probiotic are live and nonpathogenic microbes, which have a positive impact on the host animal by optimizing gut microbiota existence and modulating the immune system (Cox & Dalloul, 2015). Probiotics are microorganisms that contribute to the balance of microflora in the digestive tract by increasing the good microbial population (Sharifi *et al*., 2012; Rajoka *et al*., 2018). Indigenous probiotic candidates isolated from the digestive tract of Indonesian native chicken potentially improve performance of broiler (Harimurti & Hadisaputro, 2015). Probiotics possibly interrelate with the gut-associated lymphoid tissue and enhance immune modulating system to minimize pathogenic infection (Arena *et al*., 2018). Probiotics might be associated with the composition of their indigenous internal microbes.

Lactic acid bacteria (LAB) are commonly used as a probiotic that has ability to prevent the colonization of pathogenic bacteria on GIT and it survives on GIT especially on acid condition and bile salt (Rajoka *et al*., 2018). Acidic condition is the most constrain for probiotic viability to enter GIT. The probiotic microbe has to survive in bile salt stress in small intestine. Some studies of the LAB abilities are known to have high activity in inhibiting pathogenic bacteria and proven to survive in the gastrointestinal tract.

In the previous study, *S. cerevisiae* has a potency to reduce cholesterol accumulation in the animal product (Istiqomah *et al*., 2018). Yeast positively interacts with bacteria by producing amino acids and vitamins for stimulating bacterial growth (Hatoum *et al*., 2012). However, application of LAB and yeast as probiotics consortia is still rarely elucidated. We proposed a hypothesis that probiotic containing consortium of LAB and yeast generates synergistic effect on the host animals. This study aimed to evaluate antibacterial activity and viability of probiotics candidate consisting of *L. plantarum* AKK-30, *S. cerevisiae* B-8, and their combinations as well as the possibility interaction activity to improve energy and protein utilization of quail diet.

MATERIALS AND METHODS

In Vitro Assessment for Viability and Antimicrobial Activity

Isolate and sample preparation. Isolates used in this experiment consisted of *Lactobacillus plantarum* AKK-30 (isolated from native chicken) and *Saccharomyches cerevisiae* B-18 (isolated from Javanese duck) were molecularly identified by Julendra *et al*. (2017) and Istiqomah *et al*. (2018), respectively. Pathogenic bacteria used in the in vitro assay were *Salmonella pullorum* ATCC 13036, *Pseudomonas aeruginosa* FNCC 0063, *Staphylococcus aureus* FNCC 6049, *Escherichia coli* FNCC 0194. The selective medium for growing the isolates consisted of de Mann Rogosa Sharpe agar (MRSA) (Oxoid), chloramphenicol yeast glucose agar (CYGA) (Merck). Nutrient agar (NA) (Merck) was used as a universal medium for assaying consortia of both isolates.

In vitro assay of probiotics consisting of tolerance to acid condition, gastric juice, and bile salt were carried out according to Torshizi *et al*. (2008) and Damayanti *et al.* (2014). Acid tolerance assays were arranged on the factorial design (4 x 3 x 2) consisted of four probiotic treatments (Lp= *L. plantarum* AKK 30; Sc= *S. cerevisiae* B-8, Consortia Lp, and Consortia Sc), three incubation time (0, 45, and 90 min), and two pH medium (pH 2 and 3) with three replications. At 18-hours incubation, the media containing isolates were centrifuged with the speed of 4136 x *g* at 4° C for 10 minutes. The pellet was cleared from using PBS 2 times. Pellet suspension was added each to 990 µL sterile PBS at pH 2 and pH 3.

Antimicrobial and antibiotic sensitivity assay were conducted by using the agar diffusion method (Bonev *et al*., 2008). The antimicrobial assay was arranged in a factorial design with treatments factors (6 x 4) were antimicrobial substances (Lp, Sc, Lp+Sc, streptomycin, penicillin. and erythromycin) and pathogenic bacteria (Sp, Sa, Pa, Ec) with three replications. Antibiotic sensitivity was arranged in a completely randomized design with treatment factors (3x3); three isolates (Lp, Sc, and Lp+Sc), three antibiotics, and each treatment consisted of three replications. Antimicrobial substance of isolates was harvested from each inoculated media. After 24 h of incubation, MRSB inoculated *L. plantarum* AKK-30 (37 °C), and CYGB inoculated *S. cerevisiae* (30 °C) centrifuged at 4136 x *g* of 4 °C for 15 minutes. The supernatant neutralized with NaOH 1 M (25 µL, pH= 7) was dripped onto a blank disc paper that had been placed on the top of media of the inoculated pathogenic bacteria.

Gastric juice tolerant assay was conducted according to Torshizi *et al*. (2008). Briefly, *L. plantarum* was cultured on MRSB for 18 h at 37 °C and *S. cerevisiae* was cultured on CYGB for 18 h at 30 °C. 1 mL of each culture was centrifuged at 4136 *x g* for 10 min at 4 °C. The pellet was rinsed by sterilized PBS, diluted in 0.3 mL of PBS, mixed with 1 mL of PBS pH 2 containing pepsin 0.3% (artificial gastric juice). The cultures were sampled at 0, 15, and 45 min after incubation at 37 °C and 30 °C for *L. plantarum* and *S. cerevisiae,* respectively.

In bile salt tolerant assay, the pellet was similarly prepared with gastric juice salt tolerant assay. The diluted pellet in 0.3 mL of PBS was mixed with 1 mL PBS containing 0.3% (w/v) bile salt. The cultures were sampled at 0, 120, and 180 min after incubation at 37 °C and 30 °C for *L. plantarum* and *S. cerevisiae,* respectively. This assessment was arranged on the factorial design (4 x 3) consisted of four isolates and three observation time with three replications.

In Vivo Assay on Nutrients Digestibility

Animal, quail diet, and feed additive. Female Japanese quails (*Cortunix cortunix japonica*) at 3 weeks of age were used in this experiment. Quail's diet was formulated according to nutrient requirement of Japanese quails according to the Indonesian National Standard (SNI) No. 01-3907-2006) presented in Table 1. Probiotic *L. plantarum* was prepared by spraying-drying method (Barbosa-Cánovas *et al*., 2005) using milk skim carrier and *S. cerevisiae* B-18 was prepared by oven-dried method (AOAC, 2005) using cassava flour as a carrier.

Treatments and feeding trials. The experiment for evaluating nutrient digestibility was conducted in the Poultry Closed House, Bio-Feed Additive Technology Laboratory, Research Unit for Natural Product Technology (BPTBA), Indonesian Institute of Sciences (LIPI), Yogyakarta. The experimental protocol had been approved by the Commission of Ethical Clearance for Pre-clinical Experiment (No. 00136/04/LPPT/XI/2017), from the Integrated Laboratory of Research and Testing (LPPT), Universitas Gadjah Mada (UGM), Yogyakarta.

One hundred and fifty (150) female quails were randomly distributed into five treatments; R0) control, R1) *L. plantarum*, R2) *S. cerevisiae*, R3) R1+R2, R4) commercial probiotic. Each treatment consisted of six cages as replications and five birds in each cage. Fifteen female birds were randomly distributed in three cages as indigenous control. The experimental birds were reared and adapted from early-laying to after peak-laying period (40 d to 90 d of age) by feeding diets and drinking water according to the treatments. Measurement of nutrient digestibility was conducted by modification of the method used by Farrell *et al*. (1982). Briefly, each bird was fed quail's diet (35 g of feed per bird). The treated group was administrated probiotic by diluting in the drinking water (3.5 mg per bird). The probiotics used contained 7.8 x 10^8 cfu/g for *L. plantarum* AKK-30 and

5.3 x 10^6 cfu/g for *S. cerevisiae* B-18. The manure excreta were collected during 24 h, immediately dried at 55°C for 48 h, and packed for further chemical analysis.

Analyzing metabolizable energy and nitrogen retention. The energy content of samples (feed and manure) was estimated by the Parr ®6200 bomb calorimeter (Parr Instrument Company, USA), while crude protein of samples was analyzed by Kjeldahl methods (AOAC, 2005). Metabolizable energy (ME) and nitrogen retention (NR) were estimated according to the Sibbald & Wolinetz (1985).

Statistical Analysis

Data from *in vitro* and *in vivo* assays were statistically analyzed using analysis of variance (ANOVA). Significance difference was declared if the treatment differed at least 5%, and continued to post hoc test using Duncan multiple range test to compare differences among treatments which used Costat Statistical Software (Cohort, 2008). The logarithmic transformation was applied to convert bacteria cell count (cfu/mL) into logarithmic value of incubation (log10 cfu/mL). Part of in vitro assay parameters were retrieved from previous study (Martin *et al*., 2018) and deeply analyzed and elaborated in this experiment. In order to evaluate relationship between parameters in the *in vivo* experiment, relative value (%) of NR and ME data were analyzed using hierarchical cluster analysis (HCA) (Zhang *et al*., 2017) and visualized in dendro-heatmap by the R-statistical software (R Core Team, 2015).

Table 1. Ingredient and nutrient composition of quail's diets

Ingredients	Content (%)
Corn	55.10
Soy bean meal	30.50
Meat bone meal	4.00
Crude palm oil	2.80
Premix	0.10
DCP	0.80
Salt	0.10
Limestone	5.70
L-Lysine	0.70
DL-Methionine	0.20
Total	100.00
Dry matter (%)	88.96
Ash (%)	10.20
Crude protein (%)	17.93
Crude fiber (%)	5.36
Ether extract (%)	5.08
Nitrogen-free extract (%)	50.39
Calcium (%)	3.38
Phosphorus (%)	0.68
NaCl (%)	0.39
Gross energy (kcal kg^{-1})	3552

RESULTS

In Vitro Assessment Characteristic of Probiotic Candidate

Antibacterial activities assays of antibacterial substances of isolates and antibiotic (as a positive control) against pathogenic bacteria are shown in Table 2. Both isolates generated inhibition activity at different levels. Inhibition activity of *S. cerevisiae* was higher than that of *L. plantarum*. There was a significant interaction ($p<0.01$) between pathogenic bacteria and antibacterial substances. The highest inhibition was found in supernatant obtained from the *S. cerevisiae* B-18 against *S. aureus*, followed by those obtained from *P. aeruginosa* and *S. pullorum*. However, supernatant obtained from combination of all isolates did not inhibit *E. coli* growth (Table 2). Susceptibilities of the isolates to antibiotics showed that yeast had no clearing zone after 24 hours of incubation while *L. plantarum* had different resistance levels on each antibiotic. *L. plantarum* was categorized as sensitive microorganism to erythromycin while *L. plantarum* combined with *S. cerevisiae* showed the resistant response to all antibiotics (streptomycin, penicillin, and erythromycin) (Table 3).

Acidic condition (pH 2 and 3) and the duration of incubation significantly influenced isolates viabilities. The viability of isolate *S. cerevisiae* was higher than that of *L. plantarum*. Population of *S. cerevisiae* in pH 2 medium was lower ($p<0.05$) than that in pH 3 medium whereas populations of *L. plantarum* in pH 2 and pH 3 were similar. The viability of *S. cerevisiae* and its consor-

Table 2. Antimicrobial activities of crude bacteriocin and commercial antibiotic on pathogenic bacteria

Antimicrobial substances	Diameter of clearing zone (mm)[a]			
	P. aeruginosa	*S. aureus*	*E. coli*	*S. pullorum*
Antibiotic				
Streptomycin	11.19±2.61^{de}	11.20±0.46^{de}	6.18±0.93^{gh}	13.01±0.16^{cd}
Penicillin	15.06±3.18^{bc}	13.34±0.23^{cd}	0.00±0.00^{i}	16.76±3.49^{b}
Erythromycin	17.69±0.63^{b}	27.52±0.65^{a}	9.97±0.88^{ef}	11.90±2.15^{de}
Supernatant				
Lp AKK-30	9.84±2.98^{ef}	6.44±3.13^{gh}	0.00±0.00^{i}	7.29±0.96^{fg}
Sc B-18	15.46±1.60^{bc}	17.82±2.59^{b}	0.00±0.00^{i}	15.04±2.37^{bc}
Lp AKK-30+ Sc B-18	4.49±0.12^{gh}	4.00±0.09^{h}	0.00±0.00^{i}	4.18±0.24^{gh}

Note: *Means in the same column or row with different superscripts differ significantly ($p<0.05$).

Table 3. Antibiotic sensitivity test on the probiotic inoculated media (mm)

Isolate (Medium)	Antibiotics		
	Streptomycin	Penicillin	Erythromycin
Lp AKK-30 (MRSA)	0.00±0.00 (R)	23.11±0.73 (R)	24.18±1.04 (S)
Sc B-18 (CYGA)	0.00±0.00 (R)	0.00±0.00 (R)	0.00±0.00 (R)
Lp+Sc (MRSA)	0.00±0.00 (R)	0.11±0.01 (R)	0.43±0.07 (R)
Lp+Sc (CYGA)	0.00±0.00 (R)	0.00±0.00 (R)	0.00±0.00 (R)

Note: R: Resistant, S: Sensitive.

tia was higher than those of the others at pH 3 after 45 h of incubation (Table 4). The isolate of *L. plantarum* and their consortia were tolerant to bile salt condition during 180 minutes of incubation while *S. cerevisiae* and their consortia were viable in gastric juice during 45 minutes of incubation (Table 5).

Effect of Probiotic Inclusion on Nutrients Digestibility

Nutrient digestibility of quail's diets treated by probiotic is presented in Table 5. The values of nitrogen retention (NR) and metabolizable energy (ME) were not affected by treatments (Table 6). Quails treated by a combination of *L. plantarum* and *S. cerevisiae* were within

Table 4. Viability of probiotic isolates on the acidified culture media (%)

pH / Interaction	Incubation time / pH	Isolates			
		L. plantarum AKK-30 (Lp)	*S. cerevisiae* B-18 (Sc)	Lp+Sc Consortia (Lp AKK-30)	Lp+Sc Consortia (Sc B-18)
pH 2[x)]	0 min	100.00±0.00	100.00± 0.00	100.00±0.00	100.00± 0.00
	45	0.37±0.39	38.66± 2.48	0.69±0.07	49.69±10.54
	90	0.08±0.13	3.39± 0.48	0.34±0.04	13.02± 1.33
pH 3[x)]	0	100.00±0.00	100.00± 0.00	100.00±0.00	100.00± 0.00
	45	0.26±0.15	118.34±15.43	1.56±0.12	106.59± 5.37
	90	0.30±0.43	98.71± 7.70	2.15±0.08	89.51± 6.78
Average Isolates*Time	pH 2	0.23±0.30[c]	21.07±19.33[b]	0.52±0.20[c]	31.16±20.81[b]
	pH 3	0.28±0.29[c]	109.31±16.70[a]	1.86±0.34[c]	98.05±10.84[a]
Average pH*Isolates	45 min	0.31±0.27[b]	79.77±46.20[a]	1.13±0.49[b]	78.08±31.82[a]
	90 min	0.19±0.31[b]	50.61±51.77[a]	1.25±1.00[b]	51.13±42.27[a]

Note: [x)] pH effect indicated the significant difference, viability of probiotics in pH 3 > pH 2 ($p= 0.02$). *) Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 5. Viability of probiotic isolates on gastric juice and bile salt

Viability of isolate (%)*	Incubation time in gastric juice			Incubation time in bile salt		
	0 min	15 min	45 min	0 min	120 min	180 min
Lp AKK-30	100.0±0.0	17.9± 1.26[b]	0.09± 0.11	100.0±0.0	82.4± 1.26[b]	135.5±69.39
Sc B-18	100.0±0.0	96.0±15.76[a]	uc **	100.0±0.0	20.7± 6.46[b]	12.07± 1.88
Consortia (Lp AKK-30)	100.0±0.0	0.15± 0.11[b]	0.13± 0.05	100.0±0.0	180.2±75.73[a]	uc**
Consortia (Sc B-18)	100.0±0.0	102.5± 9.07[a]	91.5±36.47	100.0±0.0	48.9±15.81[b]	58.0± 5.76

Note: * Means in the same row with different superscripts differ significantly ($p<0.05$). ** uc denotes uncountable colony (> 300 cfu/mL). Viability data of cultures incubated after 180 minutes were excluded in statistical analysis.

Table 6. Nitrogen retention and metabolizable energy of quail diet supplemented with probiotics

Treatments	Variables					
	Retention of nitrogen (g)	Retention of nitrogen (%)	AME (kcal kg^{-1})	TME (kcal kg^{-1})	AMEn (kcal kg^{-1})	TMEn (kcal kg^{-1})
Control	183.19±26.98	54.15± 7.83	2622.59± 65.10	2885.57± 63.67	2609.82± 64.01	2872.80± 62.42
Lp	210.13±32.15	59.94± 8.76	2637.46± 59.14	2891.32± 50.71	2623.33± 58.20	2877.18± 49.41
Sc	202.43±49.11	59.48±20.38	2598.62±139.87	2857.58±146.73	2584.59±137.60	2843.55±143.74
Lp + Sc	169.10±45.09	55.49±15.60	2500.62±123.33	2790.32± 98.64	2487.54±123.99	2777.24± 98.94
Commercial	219.63±55.79	68.40±15.29	2590.22±103.88	2868.21± 91.47	2574.09±103.03	2852.08± 90.77
Average	196.89±44.22	59.49±14.26	2589.90±107.44	2858.60± 96.59	2575.87±106.63	2844.57± 95.38

Note: Control= without treatment (as negative control), Lp= *L. plantarum* AKK-30, Sc= *S. cerevisiae* B-18, Lp+Sc= combination of *L. plantarum* AKK-30 and *S. cerevisiae* B-18, Commercial= commercial probiotic consisted of *L. plantarum* and *S. cerevisiae* (as a positive control). AME= apparent metabolizable energy, TME= true metabolizable energy, AMEn= apparent metabolizable energy corrected by N-energy, TMEn= true metabolizable energy corrected by N-energy.

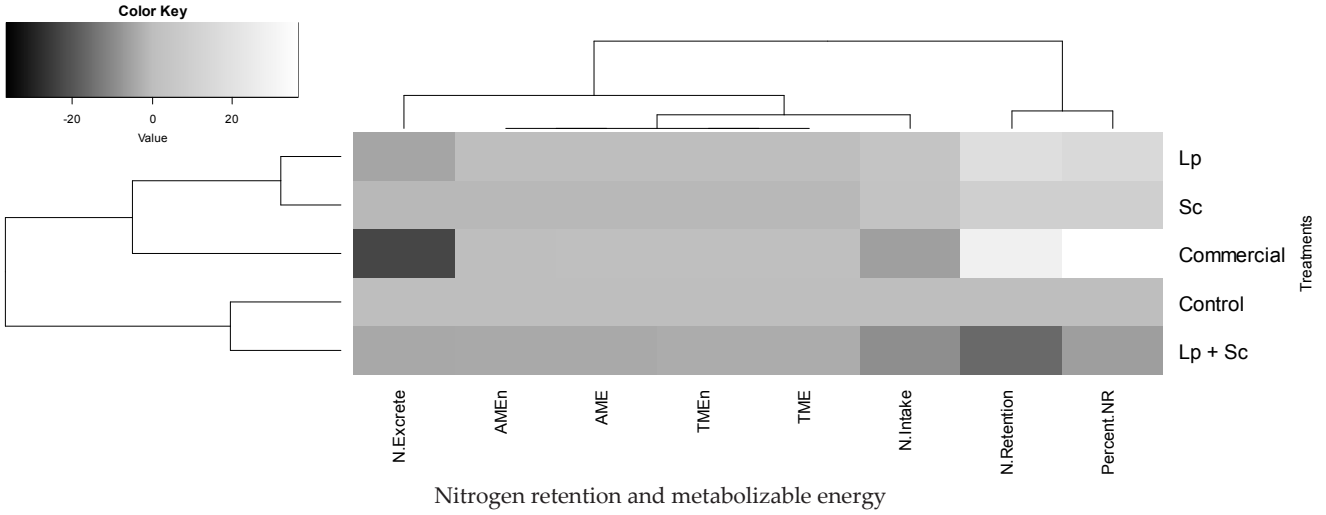

Figure 1. Dendro-heatmap visualized interrelationship between nitrogen retention and metabolizable energy influenced by the treatments. Control= without treatment (as negative control), Lp= *L. plantarum* AKK-30, Sc= *S. cerevisiae* B-18, Lp+Sc= combination of *L. plantarum* AKK-30 and *S. cerevisiae* B-18, Commercial= commercial probiotic consisted of *L. plantarum* and *S. cerevisiae* (as positive control). AME= apparent metabolizable energy, TME= true metabolizable energy, AMEn= apparent metabolizable energy corrected by N-energy, TMEn= true metabolizable energy corrected by N-energy. N-excreta= nitrogen content in excreta, N. Retention= nitrogen retention, Percent.NR= percentage of nitrogen retention.

the same cluster with the control but in a different cluster from the individual probiotics across all parameters (Figure 1).

DISCUSSION

Antibacterial activity against pathogenic bacteria, viability in low pH or bile salt, and antibiotic sensitivity are parameters that are considered in selecting probiotic candidate (de Melo Pereira *et al*., 2018). Gotcheva *et al*. (2002) reported that *Lactobacillus plantarum* and *Candida rugosa* had antibacterial activity towards *P. aeruginosa, S. enteritidis,* and *Salmonella* strains. In this study, *L. plantarum* AKK-30 and *S. cerevisiae* B-18 could inhibit *S. aureus*, *P. aeruginosa,* and *S. pullorum*. These found results indicate that both isolates have antibacterial substances. *L. plantarum* and *S. cerevisiae* secrete antimicrobial peptides (AMPs) namely plantaricin and saccharomycin, respectively, which have an important role for inhibiting pathogenic bacteria (Hammami *et al*., 2013). The mode of AMPs inhibition activity of probiotics is associated with the absorption of pathogenic bacteria on the cell-membrane receptors. The other mechanisms might correlate with the alterations of intracellular pH and membrane permeability (Hammami *et al*., 2013; Rizk *et al*., 2018).

Stanley *et al*. (2014) stated that *Lactobacillus* strain isolated from chicken possessed a high activity of antibiotics resistance. In the previous study, three selected broiler chicken-indigenous LAB had some degrees of antibiotic resistances against several antibiotics (Torshizi *et al*., 2008). The resistance of yeast isolate to antibiotic made it suitable for poultry undergoing antibiotic treatment and profitable over bacteria for therapeutic use (Syal & Vohra, 2013). In this study, *L. plantarum* and *S. cerevisiae* were resistant to streptomycin and penicillin but not to erythromycin. Damayanti *et al*. (2014) revealed that *P. acidilactici* R01 isolated from broiler's GIT, showed a sensitive response to erythromycin and resistant responses to streptomycin and penicillin.

One of the main characteristics of probiotic to give advantageous health for the host is a tolerance to the environment of GIT such as acid and bile salt (Rajoka *et al*., 2018). Bile-salt tolerance is associated with the activity of bile salt hydrolase (BSH). BSH brake down the peptide linkage of bile acids, which removes the amino acid group from the steroid core and the unconjugated bile acids precipitate at low pH (Ooi & Liong, 2010). In this study, both isolates could survive on bile salt after 3 hours. It would be possible to deconjugate bile salt and might be effective in reducing serum cholesterol in poultry. In the previous study, isolates Lp AKK-30 and Sc B-8 showed lowering cholesterol activities (Julendra *et al*., 2017; Istiqomah *et al*., 2018).

In gastric juice tolerant assay, the cell viability of isolate decreased while the yeast survived after 45 minutes of incubation. Moreover, the viability of Sc B-8 showed the highest level at pH 3 after 45 h of incubation. These results indicate that *S. cerevisiae* is tolerant of low pH. The ability of an isolate to survive in acid medium is influenced by many factors such as temperature, pH, nutrient ability, and previous natural habits (Kumar & Gopal, 2015). The viability of microorganism is closely associated with the adaptability to the environment (Sofyan *et al*., 2013).

Administration of probiotics in drinking water or broiler feed improves performance and affects the height and surface area of villi in the small intestine (Sharifi *et al*., 2012). These conditions will increase the capacity of small intestine in absorbing more nutrients. The stimulation mechanism increases digestion by probiotics in various mechanisms. Probiotics can increase the production of volatile fatty acids (VFA) consisting of acetate, propionate, and butyrate that are further

used in the tissue as energy sources for animal (Ajuwon, 2016). In this study, probiotics inclusion did not affect metabolizable energy. In contrast, Pramudia *et al*. (2013) reported that the inclusion of probiotics containing LAB increased the ME value around 2600-2875 kcal kg^{-1} by giving feed protein. Probiotics also protect epithelial cells, stimulate enzyme activity in the digestive tract, and increase nutrient absorption (Wang & Gu, 2010).

Even though nitrogen retention or metabolizable energy could be improved by the addition of *L. plantarum* AKK-30 or *S. cerevisiae* B-18, quails consuming a combination of isolates had a tendency of decreasing nutrient utilization. Furthermore, differences cluster between probiotics consortia and individual treatment might be associated with the antagonistic effects between *L. plantarum* AKK-30 and *S. cerevisiae* B-18. Antagonistic interaction might be associated with the possible mechanism i.e. competitiveness to digest the nutrients for growth (Kim *et al*., 2018). The other mechanism i.e., the cross inhibition between LAB and yeast, might be occurred. LAB produced lactic acid potentially inhibit yeast growth (Narendranath *et al*., 2001) or saccharomycin secreted by *S. cerevisiae* can inhibit bacteria (Branco *et al*. 2017). In contrast, mutual interaction between LAB and yeast could be found at sourdough (Sieuwerts *et al*., 2018) or Kefir (Stadie *et al*., 2013).

CONCLUSION

Probiotics consisting of *L. plantarum* AKK-30, *S. cerevisiae* B-18 or their combination could inhibit *P. aeruginosa*, *S. aureus,* and *S. pullorum*. Both isolates were resistant to streptomycin and penicillin, and tolerant to acids and bile salt. Inclusion of *L. plantarum* and *S. cerevisiae* did not affect the nutrient utilization of quail's diet. Further study is necessary to explore the nutrient (prebiotic) for optimizing the synergistic effect of *L. plantarum* AKK-30 and *S. cerevisiae* B-18.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest with any producers and organizations in relation to the material used in this experiment.

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