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Application of *Saccharomyces cerevisiae* as a Probiotic for Producing Low Cholesterol and Antibiotic-Free Broiler Meat

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ABSTRACT

The aim of this study was to evaluate the application of S. cerevisiae as a probiotic for producing low cholesterol and antibiotic-free broiler meat. This study used 250 broilers (14 days old) which were divided into 5 treatment groups, those were T0 (rations without *S. cerevisiae* addition), T1, T2 and T3 (rations added with 0.5, 1, and 1.5 g/kg DM of *S. cerevisiae* respectively), and K group (commercial feed). Broilers were reared until 36 days old prior to slaughter. The carcass quality, abdominal fat, internal organs, cholesterol content of breast meat, the number of LAB and *E. coli* of intestinal tract were observed. The addition of *S. cerevisiae* tended to increase the number of LAB in intestinal tract, whereas the number of *E. coli* significantly decreased. The highest slaughter weight was found in group fed with commercial feed. There were no significant effects of *S. cerevisiae* addition on carcass, abdominal fat and weight of internal and immunity organs. The lowest cholesterol content was showed on broiler fed with rations added with 1 g/kg DM *S. cerevisiae*. Therefore it could be concluded that the addition of *S. cerevisiae* up to 1 g/kg DM rations showed health improvement and able to produce low cholesterol broiler meat.

Keywords: Antibiotic-free, Broiler, Cholesterol, Probiotic, and *Saccharomyces cerevisiae*

Introduction

Chicken is the most popular food as an animal protein source in society. Its consumption globally was predicted to increase continuously over time. The study on Food Analysis Outlook of 2015-2019 by the Ministry of Trade of the Republic of Indonesia showed that chicken meat consumption in Indonesia statistically increased to 1 million tons in 2019. This number was higher than the protein consumption from other livestock (Kementerian Perdagangan, 2014). The increase of chicken meat consumption, especially for broiler meat goes hand in hand with the development of the people mindset about healthy lifestyles, including consuming healthy broiler meat, antibiotic free and low in cholesterol. In this regard, at the level of chicken meat production, the government has also implemented regulations regarding the prohibition of antibiotic usage in feed, called Antibiotic Growth Promotor (AGP). Prohibition against AGP usage in Indonesia has been regulated in Undang-Undang (UU) No. 18/2009 in conjunction with UU No. 41/2014 concerning Animal Husbandry and Animal Health, Peraturan Kementerian Pertanian No. 14/2017 concerning Classification of Animal Medicine, and also strengthened by Peraturan Kementerian Pertanian No. 22/2017 concerning Feed Registration and Circulation.

Legally prohibition of AGP usage had become a challenge for poultry farming. However, it also opens up opportunities to get healthier AGP replacement materials. One of them is probiotics, specifically *Saccharomyces cerevisiae* (*S. cerevisiae*) as a local yeast. *S. cerevisiae* was used as feed additives for probiotic agent and immunostimulant functions to improve animal health (Hussein and Selim, 2018). The advantages of *S. cerevisiae* usage as probiotics were only eliminate pathogenic bacteria and even increases the number of beneficial microbes, unlike antibiotics which killed whole microbes both harm or benefit the body, and have a resistance effect (Ahmad, 2005). Additionally, *S. cerevisiae* as probiotic were able to increase intestinal homeostasis which allows the mechanism of cholesterol degradation so that reduced cholesterol levels as study of (Trisnadewi *et al.*, 2015).

S. cerevisiae have been widely studied, especially related to its dosage about 0 to 15 g/kg DM in the poultry feed for various purposes, i.e. improving performance, egg traits, and blood profiles on laying hens (Yalçın *et al.*, 2012), carcass and gut characteristics, and antibody production on broiler chicken (Rezaeipour *et al.*,

2012; Yalcin *et al.*, 2013). However, there has not been much study with a very low dosage for its effectiveness usage as a feed additive that can reduce cholesterol levels in broiler meat and also improve animal health without AGP. Improving of broiler health were determined by the number of lactic acid bacteria (LAB) and pathogenic bacteria, specifically *Escherichia coli* in the intestinal tract, and detected by normal size of internal and immune organs of broiler chicken. Therefore, the aim of this study was to produce healthy broiler meat through improving broiler health, reducing cholesterol and producing antibiotic-free of broiler meat which fed low dosage of *S. cerevisiae* as a feed additive.

Materials and Methods

Broiler and research design

Strain Cobb broiler (PT Panca Patriot Prima), 14 days old at amount of 250 broilers with body weight around ±360 g/chick were used in this study. They were divided into 5 levels of *S. cerevisiae* with each level consisted of 5 replications, and each replication contained of 10 broilers. Treatment applied was rations without or with the addition of feed additive and commercial feed (BR1). Feed additive used in this study was probiotics in the form of yeast, specifically *Saccharomyces cerevisiae* (*S. cerevisiae*) with some administration levels. The treatment levels were:

P0 = rations without *S. cerevisiae*
P1 = rations + *S. cerevisiae* 0.5 g/kg DM (0.05%)
P2 = rations + *S. cerevisiae* 1.0 g/kg DM (0.1%)
P3 = rations + *S. cerevisiae* 1.5 g/kg DM (0.15%)
K = commercial feed (BR1).

Broilers were maintained until 36 days old, then they were slaughtered. A broiler was taken randomly from each replication per level treatment to be slaughtered. They observed on carcass, abdominal fat, relative weights of internal and immunity organs, the number of LAB and *Escherichia coli* (*E. coli*) in the intestinal tract, and breast cholesterol content.

Feed and broiler management

The rations and commercial feed were used in this study. The rations was formulated based on the broiler requirement according to Leeson and Summers (2005) with protein content of 21-22% and EM of 3.000-3.100 kcal/kg. The ingredient of rations contained ground corn, meat bone meal (MBM), rice bran, fish meal, soybean meal, coconut oil, minerals, and 'Top mix' brand (mixtures of minerals, vitamins, and amino acids). Nutrient content of each materials and the rations can be seen in Table 1, while the commercial feed (BR1) from PT Panca Patriot Prima contained moisture max 13%, protein min 20%, fat max 6%, fiber max 5%, ash max 7%, calcium 0.9 – 1.1%, and phosphorus 0.7 – 0.9%. *S. cerevisiae* as feed additive was added in the rations based on treatment levels as previously described. *S. cerevisiae* that used in this study was a local microbe in the form of yeast from the Optimet@Sc product. The product showed a concentration of 10^9 CFU/g based on the screening of previous study (Pantaya *et al.*, 2014).

Broiler that used in this study were obtained from PT Panca Patriot Prima when they were one day old chick (DOC). DOC was maintained in large brooding in the open house sized (14 x 8 x 3) m^3 with the gable type of roof. The house used was a regularly housing for broiler maintenance that has been passed the resting phase before the experiment period. DOC was fed with commercial feed and drinking water *ad libitum* up to 10 days old. Furthermore, the broilers were divided randomly into 5 levels of *S. cerevisiae*, each treatment consisted 5 replications. Each replication was represented with pen sized (1 x 1) m^2 and each pen contained 10 broilers. Broilers were also adapted of feeding for each treatment levels with successive ratios of 25%:75%, 50%:50%, 75%:25% to 100% rations before treatment period which started at day 14. The frequency of feeding were 2 times daily around 07.00 AM and 04.00 PM and drinking water was offered ad libitum.

Broilers were vaccinated using Medivac ND-IB vaccine on 5 days old and Medivac Gumboro A vaccine on 10 days old. Both vaccines were come from Medion. Vitamins administration was given routinely to reduce broiler stress levels, especially after weekly weighing and vaccination. Vitamins were only given once in the morning. Vitamins given were Vita Chick, Forte Vit, and Vita Stress based on the recommended dosage.

Carcass, abdominal fat, organs, and cholesterol content

The parameters observed by sampling on broilers harvested at 36 days old. A broiler was taken randomly for sample of each replication. Before harvesting, broilers were fasted for 4 hours with the provision of drinking water *ad libitum*. Broilers were harvested and slaughtered by observing the aspect of animal welfare. Each broiler was weighed using a hanging digital scale (Matrix Brand) before slaughtered to determine the final body weight. Broilers were hung by the position of the feet above and head down, stunned by a 600 V electric shock for 1-2 seconds, then slaughtered on the jugular vein where blood could be discharged as much as possible. Then, broilers slaughtered were soaked in 55-60^{o}C of water for 3-5 minutes then scalded using a thresher machine.

Broiler carcasses which whole contents of the abdominal cavity, head and legs had been removed, were weighed as carcass yield. Abdominal fat weight was obtained from all fat contained in the abdominal cavity including attached to the organs. Internal organs consisting of gizzard, heart and liver, and immunity organs consisting of spleen and bursa fabricius were weighed separately. The gizzard weight was obtained after cleaning the entire contents inside.

The relative weight of carcass yield, abdominal fat and each organ were calculated with the following formula:

$$\text{The percentage of X (\%)} = \frac{\text{weight X (gram)}}{\text{final body weight (gram)}} \times 100$$

Note: X = carcass, abdominal fat, and body organs.

Cholesterol content of broiler breast meat were obtained from cholesterol analysis through the Liebermann–Burchard method (Kleiner and Dotti, 1962) at the Laboratory of Nutrition Biochemistry, Faculty of Animal Science, University Gadjah Mada. The sample of broiler breast meat (about 1 g) was extracted with organic solvents (ether alcohol) then centrifuged at 3,000 rpm for 10 minutes to obtain a supernatant. The supernatant was heated at 100 ^{o}C in a 50 ml beaker glass up to dry and the solution was used up. The residue was extracted into a scale tube with chloroform added to reach 5 ml. The standard of cholesterol (5 ml) and chloroform (5 ml) for the blank were put into two other glass tubes. The standard cholesterol used was 4 mg/5ml. As much as 2 ml of anhydrous acetic acid and 0.2 ml of concentrated sulfuric acid were added to the 3 tubes then allowed to stand for 10 minutes. They were read using spectrophotometer with 420 nm wavelength. Cholesterol levels were calculated using the following formula:

$$\text{Cholesterol (mg\%)} = \frac{\text{sample absorbance}}{\text{standart absorbance}} \times \frac{\text{standart concentration}}{\text{sample weight}} \times 100\%$$

Analysis of the number of lactic acid bacteria (LAB) and *E. coli* in the intestinal tract

The number of LAB and *E. coli* in the broiler intestinal tract were calculated using the modified pour plate method from Wulandari *et al.* (2014). A gram digesta sample in the intestinal tract for each treatment was taken and put into 9 ml of physiological saline solution then homogenized until a 10^{-1} dilution. Dilution of the sample was repeated from the 10^{-1} dilution until it reaches the intended concentration. The concentration of dilution was 10^{-9} and 10^{-6} for LAB and *E. coli*, respectively. A ml of each dilution was taken to inoculate using agar media into a petri dish. MRS and EMB agar as the selective media were used to analyze the number of LAB and *E. coli*, respectively. The LAB inoculation was incubated aerobically while *E. coli* was incubated anaerobically for 48 hours at 37^{o}C. When the incubation period was complete, the colonies growth of LAB and *E. coli* in each petri dish were counted. The total number of microbes was determined based on the number of growth colonies and calculated using the following formula:

$$\text{The total number of microbes (CFU/ml)} = \text{number of colonies each patridish} \times \frac{1}{\text{concentration of dilution}}$$

Statistical analysis

The percentage of carcass yield, abdominal fat, internal and immunity organs, and cholesterol content of breast meat parameters using a completely randomized design (CRD) with as following model:

$$\gamma_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Note:

γ_{ij} = observation value of i-treatment and j-replication

μ = grand mean value

τ_i = the effect of i-treatment

ε_{ij} = the error effect of i-treatment and j-replication.

Whereas, the number of LAB and *E. coli* in the intestinal tract were counted by using a randomized block design (RBD) with 5 treatments and 3 groups of different sampling times and as following model:

$$\gamma_{ij} = \mu + \delta_i + \tau_j + \varepsilon_{ij}$$

Note:

γ_{ij} = observation value of i-treatment and j-replication

μ = grand mean value

δ_i = the effect of i-treatment

τ_j = the effect of j-group

ε_{ij} = the error effect of i-treatment and j-group

Table 1. Nutrient content of feed ingredients and the rations

Feed ingredients	Rations composition (%)	Nutrient content				
		DM (%)	CP (%)	EE (%)	CF (%)	ME (Kcal/kg)
Corn	60	87.8	8.2	3.8	0.6	2880
MBM	20	94.2	55.73	2.1	2	3500
rice bran	7	90.6	13.6	15	3	2887
Fish meal	3	89.5	63.3	4.7	2.2	2688
Soybean meal	6	87.1	49	1	2.6	2489
Coconut oil	2	-	-	4.1	-	8600
Mineral	1	-	-	-	-	-
Top Mix	1	-	-	-	-	-
Rations*	100	89.77	21.86	4.033	1.192	3032

Note:

* = rations from feed formulation.

MBM = meat bone meal; DM = dry matter, CP = crude protein; EE = ether extract; CF = crude fiber; ME = metabolic energy.

Data were analyzed by analysis of variance (ANOVA) using IBM SPSS Statistics 23. The significant results were followed by Duncan's new multiple range test (DMRT) to determine differences between treatments.

Results and Discussion

The effect of *Saccharomyces cerevisiae* as feed additive on the number of lactic acid bacteria and *E. coli* in the intestinal tract of broiler chicken

There were various effects of probiotics in nutrition. It was possible because the efficiency of probiotics depends on several factors, such as the administration level, application method, feed nutrition, chicken age, and environmental stress factors (Eltazi *et al.*, 2014). The addition of *S. cerevisiae* as feed additives on antibiotic-free rations tend to increase the number of LAB in the intestinal tract (P1 and P2). The number of *E. coli* in intestinal tract significantly decreased as same as in the broiler which fed with commercial feed. The number of lactic acid bacteria (LAB) and *E. coli* could be seen in Table 2.

S. cerevisiae as feed additive took a role in improving animal health, specifically as a probiotic and immunostimulant function. Instead of killing the pathogenic microbes, *S. Cerevisiae* increased the number of beneficial microbes (Ahmad, 2005; Kompiang, 2009). Contrary, the function of antibiotic was killing the harm or beneficial microbes and having resistance effect (Etikaningrum and Iwantoro, 2017). It was supported by 0% broiler mortality without being drugs offered at all and antibiotic-free rations as well during broiler maintenance.

The addition of *S. cerevisiae* for 0.05% and 0.15% levels significantly reduced the number of *E. coli* in the broiler intestinal tract. It might be caused acidic environmental of intestinal tract due to LAB development in the digestive system. This acidic condition was not suitable for the growth of *E. coli*. In the normal digestive environmental, both pathogens and beneficial microorganisms were in balanced condition. Also, there was some interactions such as symbiosis and competition between the two microorganisms. The balance of the intestinal microorganisms will be achieved when beneficial microbes can suppress the existence of harmful or pathogenic microbes.

The effects of *Saccharomyces cerevisiae* as feed additives on the percentage of carcass yield, abdominal fat, and cholesterol of broiler meat

The percentage of carcass yield, abdominal fat, and cholesterol of broiler meat that fed antibiotic-free rations with or without *S. cerevisiae* as feed additive for 14-35 days could be seen in Table 3. Analysis of variance on harvest broiler weight showed significantly different ($P<0.05$) between treatment levels. The harvest broiler weight with commercial feed presented the highest value. Whereas, broiler fed rations containing *S. cerevisiae* showed the same value of harvest broiler weight (around 1.828 g/chick) even though it tended to increase with the increasing level of *S. cerevisiae* usage.

The addition of *S. cerevisiae* in rations presented no significant effect on carcass percentage. This condition was in accordance with previous researcher, Cengiz *et al.* (2015) which showed that yeast supplementation had no effect on body weight and carcass production. It was caused by the yeast administrations have not significantly affected the intestinal microflora which also play a role on health improvement and absorption of feed nutrient in the intestinal tract. Described by Yalcın *et al.* (2013) that improving intestinal health will increase the absorption and utilization of feed nutrients. The results of this study indicated that broilers fed with antibiotic-free rations containing the addition of *S. cerevisiae* to 1.5 g/kg of DM rations were more instrumental on improving health or immunity status (with 0% mortality) rather than increasing carcass production. It was indicated by the increase of intestinal microflora mainly LAB which was not significantly different and decrease of *E. coli* as pathogenic microbes such as data in Table 4 and the previous explanations.

Table 2. The number of lactic acid bacteria (LAB) and *E. coli* of broiler intestinal tract

Parameter	Unit	P0	P1	P2	P3	K
LAB^{ns}	CFU/ml	($8.37x10^7$)	($4.15x10^8$)	($1.79x10^9$)	($9.97x10^7$)	($6.22x10^8$)
E. coli	CFU/ml	($3.89x10^6$)^b	($7.77x10^5$)^a	($4.04x10^6$)^b	($1.03x10^6$)^a	($4.77x10^5$)^a

^{abc} different letters on the same line shows different results ($p<0.05$)
^{ns} non-significant
P0 = rations (control) without *S. cerevisiae* addition, P1 = control with *S. cerevisiae* 0.5 g / kg DM (0.05%), P2 = control with *S. cerevisiae* 1 g / kg DM (0.1%), P3 = control with *S. cerevisiae* 1.5 g / kg DM (0.15%), and K = commercial feed.

Table 3. Relative carcass yield, abdominal fat, and cholesterol of broiler meat

Parameter	Unit	P0	P1	P2	P3	K
Harvest weight	g/chick	1764^a	1788^a	1876^a	1884^a	2305^b
Carcass^{ns}	%	63.57	65.38	64.67	66.89	66.93
Abdominal fat^{ns}	%	1.7	1.83	1.95	2.06	1.58
Cholesterol content of breast meat	mg/100g	73.23^{bc}	68.89^b	60.65^a	70.2^b	79.45^c

^{abc} different letters on the same line show different results ($p<0.05$)
^{ns} non-significant
P0 = rations (control) without *S. cerevisiae* addition, P1 = control with *S. cerevisiae* 0.5 g / kg DM (0.05%), P2 = control with *S. cerevisiae* 1 g / kg DM (0.1%), P3 = control with *S. cerevisiae* 1.5 g / kg DM (0.15%), and K = commercial feed.

Some influenced factors were carcass production in broiler with probiotics including the doses and method of administrations, feed ration composition, broiler age, and the management (Cengiz *et al.*, 2015).

The administration of *S. cerevisiae* to 1.5 g/kg of DM rations indicated no different effect on broiler abdominal fat. The results of Yalcın *et al.* (2013) indicated that administration of *S. cerevisiae* would significantly reduce abdominal fat at doses 2 and 3 g/kg of dry matter feed. It could be caused by the excess of energy did not store into body fat, but it used to increase the body's immunity or immune system. According to Shurson (2018), the processing of feed into pellet required more attention because the heat generated during pelleting could kill the microbes. They did not survive during pelleting due to the denaturation of the enzyme system so that microbial activity became disrupted then died. *S. cerevisiae* became ineffective when heated at 85^{o}C with 7% of humidity for 8 minutes.

Cholesterol content of broiler breast meat were significantly lower with *S. cerevisiae* as feed additive in the rations compared to broiler fed with rations without containing *S. cerevisiae* and commercial feed. The lowest cholesterol content was presented on broiler fed with rations containing *S. cerevisiae* of 1 g/kg DM rations (Table 5). According to Trisnadewi *et al.* (2015), the usage of *S. cerevisiae* as feed additive reduced cholesterol content by converting cholesterol to cholate bile acids. The effect of yeast on the reduction of cholesterol content in broilers occurred by the crystallization of cholesterol in the bile to a gallstone (Onwurah *et al.*, 2011). Yeast also caused the increasing intestinal bile salts which can prevent 3-hydroxyl-3-methylglutaryl-coenzyme reductase as the precursors in cholesterol synthesis (Hussein and Selim, 2018), so that cholesterol content of broiler chicken would be decreased.

The effect of *Saccharomyces cerevisiae* as feed additives on the relative weights of internal organs and immunity organs of broiler

The internal organs measured in this study included gizzard, heart, and liver while the immunity organs included spleen and bursa fabricius. The mean of relative weight of broilers internal organs and immunity organs could be seen in Table 4.

The relative weight of gizzard on 36 days old broiler ranged from 1.02 to 1.22% and the results of variance analysis showed no differences on it. Range of these values was smaller than the study Hussein and Selim (2018) was around 1.5%. It was probably caused by differences on broiler strain and the types of feed ingredients. Gizzard as one of the main organs in the digestive system of birds which play a role in the process of mechanical digestion.

S. cerevisiae did not affect the relative weight of broiler heart. In this study, the mean of relative weight of heart ranged from 0.48-0.55%. Putnam (1991) presented that the normal relative weight of broiler heart was in range of 0.42-0.70%. Similar results in the normal range between 0.42-0.70% were also obtained in the studies of Hussein and Selim (2018), Shareef and Al-Dabbagh (2009), Paryad and Mahmoudi (2008). Visually, the broiler heart showed no abnormalities or swelling condition. The heart showed a good role in blood circulation. The addition of *S. cerevisiae* as much as 5-15 g/kg DM feed did not have a negative impact on the working mechanism of broiler heart.

Relative weight of the liver did not change with *S. cerevisiae* addition. It was around 2.17%. This value was within the range of the results of Hussein and Selim (2018) which were 2.13-2.22% but it was below the range of relative weight of normal liver according to Putnam (1991) which was 2.64-3.3%. Liver is a vital organ in the metabolism of nutrients, especially fat and becomes the largest gland in the body that produces acids and bile salts. The visual shape of the liver in this study look normal and there was no indication of liver enlargement. The enlargement indicated excessive work. It can be caused by toxins or antinutrients or too high fat content in the feed.

Rations without or with the addition of *S. cerevisiae* or commercial feed did not affect the relative weight of spleen and bursa fabricius. The range of relative weight of spleen and bursa fabricius in this study were 0.09-0.13% and 0.04-0.07%, respectively. Relative weight of spleen was similar to the study results of Nkukwana *et al.* (2014) which was around 0.09-0.11%. While, the relative weight of bursa fabricius was smaller than Toghyani *et al.* (2010) which was around 0.09% on 42 days old broiler.

Table 4. Relative weights of internal organs and immunity organs of broiler

Parameter	Unit	P0	P1	P2	P3	K
Internal organ:						
Gizzard	%	1.22	1.22	1.15	1.18	1.01
Heart	%	0.55	0.51	0.53	0.55	0.48
Liver	%	2.09	2.41	1.96	2.25	2.16
Immunity Organ:						
Spleen	%	0.13	0.12	0.09	0.10	0.11
Bursa fabricius	%	0.07	0.06	0.04	0.05	0.05

[abc] different letters on the same line show different results ($p < 0.05$)

[ns] non-significant

P0 = rations (control) without *S. cerevisiae* addition, P1 = control with *S. cerevisiae* 0.5 g / kg DM (0.05%), P2 = control with *S. cerevisiae* 1 g / kg DM (0.1%), P3 = control with *S. cerevisiae* 1.5 g / kg DM (0.15%), and K = commercial feed.

The difference in value can be caused by differences in strain and age of broiler and the treatment used. Spleen and bursa fabricius were lymphoid organs that become a part of the bird immunity system. Visually, the shape and size of the spleen and bursa fabricius in this study were considered normal. It showed that the broiler immune system was good and also supports the broiler health improvement.

Conclusion

The results of this study indicate that rations without or with the addition of *S. cerevisiae* as the feed additives in broiler up to 1.5 g/kg of DM rations (0.15%) showed a tendency to increase the livestock health status. It was indicated by the tendency of increasing body immunity through increasing growth of lactic acid bacteria and decreasing the number of *E. coli* in the broiler intestinal microflora, as well as the shape and size of organs were normal. *S. cerevisiae* of 1 g/kg DM rations as feed additive was able to reduce the cholesterol content of breast broiler meat to the lowest number compared to other treatments.

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