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# Effects of jamu makarens in the ration on performance and the number of pathogenic microbes in the intestines of broiler chickens

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## Abstract
Research that aimed to examine the effect of *jamu* makarens in the rations on performance and intestines pathogenic microbes has been carried out for 35 days using 80 head broilers. A completly randomized design with four treatments and four replications was used in this study. The treatments were: Commercial rations without *jamu* makarens (A), Commercial rations + 2% *jamu* makarens (B), Commercial rations + 4% *jamu* makarens (C), and Commercial rations + 6% *jamu* makarens (D). The results showed that feed and water consumptions, feed convertion ratio (FCR) and pathogenic microbes in the intestines of broilers fed 2-6% *jamu* makarens lower than control ($P<0.05$), but final body weight and body weight gain not significantly affect. It was concluded that the administration of 2-6% *jamu* makarens in the rations could reduce feed consumption, drinking water, FCR and pathogenic bacteria in the intestines, but did not effect on final body weight and body weight gain.

**Keywords:** broiler chickens, *jamu* makarens, pathogenic microbes, performance

## Introduction
Broilers are a breed of chickens (*Gallus gallus domesticus*) that are bred and raised specifically for meat production. The advantages of broiler chickens are having a high growth rate and feed efficiency so that it can be marketed/harvested at the age of 4-5 weeks with a body weight of 1.5-2 kg. To achieve maximum productivity, broiler chickens need good feed quality and environmental conditions. Good feed quality will be optimally utilized by livestock if the health condition of the digestive tract is also good, because this is where the process of digestion and absorption of food substances occurs. The process of absorption of food substances takes place in the walls of the small intestine that have villus to expand the surface. The health of the small intestine, one of which is influenced by the population of microbes in it, including bacteria. There are beneficial bacteria, such as lactic acid bacteria and there are also pathogenic bacteria, namely *Salmonella* and *Coliform* bacteria such as *E. coli* (Halimatunnisroh, 2017) [6]. To control the growth of pathogenic bacteria, it is usually done by adding antibiotic growth promoters (AGP) to the feed. However, this is dangerous because it causes residues in livestock products and when consumed by humans can cause antibiotic resistance (Anggitasari *et al*., 2016) [1].
One way to maintain the gastrointestinal tract health without any side effects is to consume a natural herbs such as "*jamu* makarens ". *Jamu* is a term for traditional medicine from Indonesia, later it was popularly known as herbal. *Jamu* makarens is a fermented herbal medicine made from ripe maja fruit (*Aegle marmelos* L.), old coconut water, palm sugar, and rice washing water. This herbs contain phytochemical compounds such as alkaloids, flavonoids, saponins, terpenoids, phenolics and tannins. According to Julianto (2009) [8], phytochemical compounds have antibacterial, anti-inflammatory and antioxidant properties. This herbs also contain lactic acid bacteria that function as probiotics.
Currently, research on the use of probiotics as a substitute for Antibiotic Growth Promoter (AGP) is being intensively carried out. Mahardika *et al*. (2019) [11] report that herbal probiotics are able to replace the use of zinc bacitracin as AGP. It is also reported that the use of drinking water-soluble probiotics and spray litter can improve the performance of broiler chickens because it is able to reduce feed convertion ratio values and mortality. Probiotics can also lower blood serum cholesterol levels and spleen weight, increase blood haemoglobin, illeum

small intestine weight, jejenum and ileum length. Furthermore, Simorangkir *et al*. (2020) [14] reported that the method of administering probiotics (through ration, drinking water, and force-feeding) in broiler chickens has no effect on body weight gain and pH of the small intestine, but can increase total lactic acid bacteria and lower *Coliform* in the small intestine. In addition to the method of administration, the dose of probiotics also greatly affects its effectiveness in livestock. According to Susinarla (2016) [19] it was found that a dose of 4% in the ration has the best influence on growth and FCR.

Based on this information, this study needs to be carried out to determine the effect of giving *jamu* makarens in the ration on the performance and number of pathogenic bacteria (*E. coli*, *Coliform* and *Salmonella*) in the intestines of broiler chickens aged of 35 days.

## Material and Methods

### Experimental design, animals, housing and diets

A total of 80 broilers were kept in battery colony cages for 35 days. The ration given is a commercial ration supplemented by *jamu* makarens according to the treatment. The experimental design used was a completly randomized design (CRD) consisting of four treatments and four replications. The treatments were: broilers fed commercial rations without *jamu* makarens (A), broilers fed commercial rations + 2% *jamu* makarens (B), broilers fed commercial rations + 4% *jamu* makrens (C), and broilers fed commercial rations + 6% *jamu* makarens (D).

### Preparation of *jamu* makarens

*Jamu* makarens are made from several ingredients i.e, ripe maja fruit (*Aegle marmelos* L.), old coconut water, palm sugar and rice washing water. Before mixing, ripe maja fruit is blended first until it is in the form of juice and palm sugar is diluted by heating then left until it cools. After that, all the ingredients are put in a barrel and fermented naturally for a month. After a month, the makarens herbs are filtered and ready to be given to the broiler. The administration of jamu makarens is carried out by mixing in the ration according to the treatments.

### Performance

The performance of broiler observed in this study i.e., feed and drinking water consumption, initial body weight, final body weight, body weight gain, and feed conversion ratio (FCR). Feed and drinking water consumption is calculated during the study (35 days). Feed consumption is measured weekly by reducing the amount of feed given with the residual feed than totaled for 5 weeks. Drinking water consumption is measured daily by reducing the amount of drinking water given with residual than totaled for 35 days. Initial body weight measured at the beginning of the study using digital scales. Final body weight is carried out at the end of the study. Body weight gain is determined by reducing the final body weight by the initial body weight. Body weight gain measured weekly, before weighing chickens are fasted for 12 hours to empty the contents of the gastrointestinal tract. Feed Conversion Ratio (FCR) is a comparison between feed consumption and weight gain.

### Pathogenic Microbes (*E.coli, Colliform*, and *Salmonella*)

The amount of *E.coli*, *Colliform*, and *Salmonella* in the small intestine is carried out by the dispersal method. The number of bacteria is calculated after the medium is incubated for 24 hours at a temperature of 37 $^{0}$C. The fecal sample was picketed as much as 0.1ml using a micro pipette ranging from dilution $10^6$ to dilution $10^8$ to be inoculated on MRS Agar media. The inoculation process is carried out inside the laminar air flow cabinet. The petri dish is inoculated in the incubator for 48 hours with a temperature of $37^{0}$C in an inverted state with the aim of avoiding water droplets. Colonies of growing bacteria are calculated using colony couters. The total bacteria are expressed in the cfu/ml log and can be calculated by the following formula:

$$\text{Total bacteria/ml} = \text{Total coloni} \times \frac{1}{\textit{Dilution factor}} \times 10$$

### Statistical analysis

All data were analyzed using one-way ANOVA to determine the differences among treatments. If differences were found ($p$<0.05), then further analysis was performed with Duncan's multiple range test (Steel and Torrie, 1993) [15].

## Results

The administration of *jamu* makarens in the ration at the level of 2-6% in general shows satisfactory results. This herbal medicine is very good for suppressing pathogenic bacteria in the small intestine, so that it has the potential to be a substitute for antibiotics. Data on the effect of administration *jamu* makarens in the rations on performance and pathogenic microbe (*E coli*, *Colliform* and *Salmonella*) in small intestine of broiler chickens age of 35 days is shown in Table 1.

**Table 1:** The effect of *jamu* makarens on performance and pathogenic microbes in the intestine of broiler chickens

| Variable | Threatmens$^{1)}$ | | | | SEM$^{2)}$ |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Feed consumption (g/h/5 weeks) | 2831.35$^b$ | 2708.05$^a$ | 2683.75$^a$ | 2645.05$^{a3)}$ | 23.50 |
| Water consumption (ml/h/5 weeks) | 7985.20$^b$ | 6800.80$^a$ | 6414.35$^a$ | 6596.45$^a$ | 4.78 |
| Initial body weight (g) | 38.70$^a$ | 38.70$^a$ | 38.65$^a$ | 38.65$^a$ | 0.18 |
| Final body weight (g) | 1807.71$^a$ | 1817.92$^a$ | 1868.54$^a$ | 1873.63$^a$ | 36.96 |
| Body weight gain (g/5 weeks) | 1769.01$^a$ | 1779.22$^a$ | 1829.89$^a$ | 1834.98$^a$ | 36.95 |
| *Feed convertion ratio (FCR)* | 1.60$^b$ | 1.52$^a$ | 1.47$^a$ | 1.44$^a$ | 0.03 |
| *E.coli* (CFU/g) | 1375.00$^b$ | 452.50$^a$ | 323.13$^a$ | 331.25$^a$ | 63.27 |
| *Coliform* (MPN/g) | 8200.00$^b$ | 610.00$^a$ | 176.25$^a$ | 170.63$^a$ | 15.25 |
| *Salmonella* (CFU/g) | 143.75$^b$ | 15.63$^a$ | 14.38$^a$ | 7.50$^a$ | 9.67 |

**Notes**

1) Treatments

A = Broilers fed commercial rations without *jamu* makarens (control)

B = Broiler fed commercial rations + 2% *jamu* makarens

C = Broiler fed commercial rations + 4% *jamu* makarens

D = Broiler fed commercial rations + 6% *jamu* makarens
2) SEM = Standar Error of the Treatment Means
3) Values with different superscripts on the same line indicate a significantly different ($p$<0.05)

The data in Table 1 shows that the administration of *jamu* makarens in the ration at the level of 2-6% has a significantly effect ($p$<0.05) on the feed consumption. Feed consumption in treatment A was 2831.35 g/head/35 days, but in treatment B (the admintration of 2% *jamu* makarens), treatment C (the admintration of 4% jamu makarens) and treatment D (the admintration of 6% jamu makarens) significantly ($p$<0.05) decrease by 4.35%, 5.21%, and 6.58% respectively compared to the control (A). Feed consumption among the treatments that fed *jamu* makarens (B, C, and D) showed not significanly defferent ($p$>0.05) between the three treatments. Water consumption also decreased significantly ($p$<0.05) by 14.83% in treatment B; 19.67% in treatment C and 17.51% in treatment D compared to the control (A). The final body weight and body weight gain values tended to be higher compared to the controls, but statistically not significantly differed ($p$>0.05). The FCR value decreased significantly (P<0.05) by 4.99% in 2% administration (B), 8.49% in 4% administration (C), and 9.99% in 6% administration (D) compared to the controls. The number of pathogenic microbes (*E.coli, Coliform* and *Salmonella*) in the intestines of broiler chickens that fed *jamu* makarens decreased significantly (P<0.05) in line with the increase of the levels given. The amount of *E.coli* decreased by 67.09% in 2% administration (B), 76.50% in 4% administration (C) and 76.63% in 6% administration (D). The amount of *Coliform* a relatively large decrease, which was 97.85% in the B treatment; 97.85% in C treatment and 97.92% in D treatment. Decrease in S*almonella* amount is also relatively large, which is 89.13% in treatment B; 89.99% in treatment C and 94.78% in treatment D compare to the controls (A).

## Discussion

The administration of *jamu* makarens as much as 2-6% in the ration turned out to cause a significantly decrease in feed consumption of broiler chickens compared to the controls. This is because of the rations containing *jamu* makarens are fermented by lactic acid bacteria in the *jamu* makarens, so that they are easier to digest. Thus the nutrients available to chickens, especially energy, will be fulfilled faster so that chickens eat less. In general, chickens eat to meet their energy needs. If the energy needs of the chickens are sufficient, the chicken will stop consuming feed even though the digestive tract is still able to accommodate the feed. However, if the ration is not energy dense (high in fiber) then the capacity of the digestive tract is a limiting factor. In addition, lactic acid bacterial as probiotics in the *jamu* makarens are able to convert carbohydrates into lactic acid and are used by chickens as a source of energy. The results of this study are in accordance with Astuti (2015) [4] which reports that the addition of liquid probiotics in the ration can reduce feed consumption, protein consumption, feed conversion, mortality and increase weight gain, as well as the weight and percentage of broiler carcasses.

Drinking water consumption decreases in line with the number of feed consumed by broiler chickens. This is in accordance with Antara *et al*. (2019) [2] which states that feed consumption is directly proportional to the consumption of drinking water, because drinking water is very necessary to dissolve food substances. Water plays an important role in metabolic processes, softens feed during the digestive process, and serves to cool the body. Lack of water in chickens cannot be underestimated because it will lead to imperfect or stunted growth of chickens and can even lead to death.

The administration of jamu makarens in the ration at the level of 2-6% has not been able to significantly increase the final body weight and body weight gain (P<0.05), but from the data obtained, the value tends to be higher than the control. The nonsignificant effect of *jamu* makarens is thought to be caused by a more active nature in chickens that consume *jamu* makarens. The chickens look more agile, close to the nature of native chickens so that a lot of energy is wasted on moving. This shows that chickens who are given *jamu* makarens have stronger conditions than those who do not consume *jamu* makarens.

In terms of the feed efficiency, broilers that consume *jamu* makarens have a lower FCR value ($p$<0.05) compare to the control. FCR is a comparison between the number of rations consumed and the increase in body weight in the same unit of time. The FCR value is an indicator for assessing the degree of efficiency of ration use in livestock. The lower the FCR value, the more efficiently the livestock uses rations, and vice versa (Sumadi, 2018) [16]. Rapid growth with a small amount of ration consumption indicates a high efficiency of feed use. The administration of *jamu* makarens in the ration was able to significantly reduce the FCR value of broiler chickens ($p$<0.05) by 7.43% in the administration of 2% (B), 10.97% in the administration of 4% (C), and 10.75% in the administration of 6% (D). This suggests that *jamu* makarens are quite effective in increasing the feed efficiency of broiler chickens.

The decrease of FCR value in this study is caused by the presence of lactic acid bacteria that produce digestive enzymes such as: amylase, protease and lipase which can increase the concentration of digestive enzymes in the broiler digestive tract so that nutrient digestibility can be improved and nutritional substances such as fats, proteins, and carbohydrates that are usually wasted a lot in faeces will be reduced (Jin *et al*., 1997) [7]. In addition, lactic acid bacterial probiotics are also able to suppress pathogenic bacteria in the small intestine (Table 1), so that the health of the intestinal villi is maintained and the absorption of nutrients is optimal. This result is in accordance with the research of Kompiang (2009) [10] which explains that probiotics can increase the activity of digestive enzymes so that food absorption becomes more perfect with the wider absorption area, because probiotics can affect the intestinal anatomy, namely the intestinal villi becomes longer and the density is denser. The surface of the villi that has many microvili is where the absorption process of digestion results takes place.

The administration of jamu makarens in the ration at the level of 2-6% caused significantly effect on reducing the number of pathogenic bacteria such as *E. coli, Coliform* and *Salmonella* in the intestines of broiler chickens (Table 1). A comparison of the amounts of *E. coli, Coliform* and *Salmonella* in the intestines of broiler chickens is shown in Figure 1.

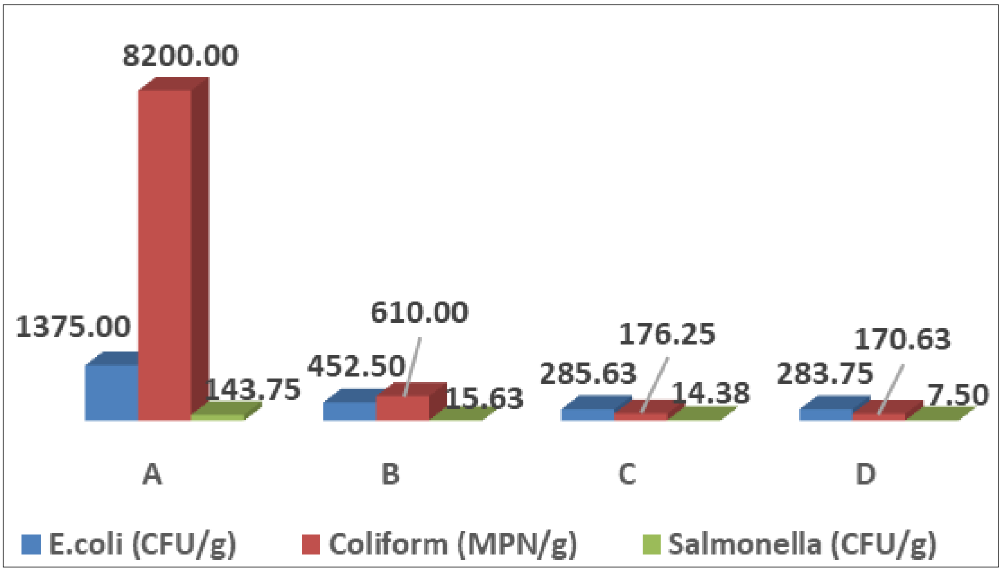


**Fig 1:** Comparison of the amount of *E. coli, Coliform* and *Salmonella* in the intestines of broiler chickens.

The decrease in the number of *E. Coli*, *Coliform* and *Salmonella* bacteria reflects that *jamu* makarens is very effective for suppressing the growth of pathogenic bacteria in the digestive tract (intestines) of broiler chickens. This is because of *jamu* makarens contains phytochemical compounds, such as alkaloids, phenolics, terpenoids, flavonoids, saponins, and tannins. This compound has biologically active properties so it is also known as a bioactive compound. For animals and humans, this compound has benefits as an antibacterial, anti-inflammatory and antioxidant (Julianto, 2019) [8]. In addition to phytochemical compounds, *jamu* makarens also contain lactic acid bacteria that function as probiotics. Probiotics are live microbes that are used as feed additives that provide benefits for animal and human health by maintaining microbial balance in the intestines (Savita *et al.*, 2021) [13].

Alkaloids have antibacterial abilities by disrupting the constituent components of peptidoglycan in bacterial cells, so that the layers of the cell wall are not formed intact and cause the death of these cells. In addition, in alkaloid compounds there are alkaline groups containing nitrogen will react with amino acid compounds that make up the bacterial cell wall and bacterial DNA. This reaction results in changes in the structure and arrangement of amino acids which will cause changes in the genetic balance of the DNA chain so that it will be damaged and encourage bacterial lysis which will cause cell death in bacteria (Arlofa, 2015) [3].

The mechanism action of phenol compounds as antibacterials is to lyse cells and cause protein denaturation, inhibit the formation of cytoplasmic proteins and nucleic acids and inhibit ATP-ase bonds on cell membranes (Sasaki *et al.*, 2003) [12]. The mechanism of triterpenoids as antibacterial is to react with purines on the outer membrane of bacterial cells to form strong polymer bonds resulting in the destruction of purines. The destruction of purines which are the entrance and exit of compounds will reduce the permeability of bacterial cell membranes which will result in bacterial cells lacking nutrients and will inhibit bacterial growth or death (Arlofa, 2015) [3].

Flavonoids are good reducing compounds, inhibiting many oxidation reactions both enzyme and non-enzyme. Flavonoids are widely used as medicinal ingredients because they exhibit antimicrobial, antiviral, antioxidant, antihypertensive activity, stimulate the formation of estrogen and treat impaired liver function (Jung *et al.*, 2006) [9]. The action mechanism of flavonoids as antibacterials by forming complex compounds against extra cellular proteins that cause denaturation of bacterial cell proteins so that cell membranes are damaged. Flavonoids are a group of phenol compounds that have a tendency to bind to proteins, thereby disrupting the metabolic processes of bacteria. In addition, flavonoids also function as antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes. Flavonoids have a chemical structure in the form of beta rings and OH-groups which are suspected to be responsible for antibacterial activity (Arlofa, 2015) [3].

Saponins have antibacterial activity with a mechanism of action, that is, it causes the leakage of proteins and enzymes from inside the cell. This can happen because the active substances contained on the surface of the saponin are similar to detergents, as a result of which the saponins will lower the surface tension of the bacterial cell wall and damage the permeability of the membrane. Then the saponins diffuse through the outer membrane and cell wall so that it interferes with and reduces the stability of the cell membrane. This causes leakage and cytoplasm out of the cell resulting in the death of Gram-negative and positive bacterial cells (Suresh *et al.*, 2013) [17].

The antibacterial properties of tannins are thought to be due to their toxicity can damage bacterial cell membranes. Tannins can induce the formation of complexes tannin bonds to metal ions that can increase their toxicity. The mechanism of action of tannins is thought to be able to shrink cell wall or cell membranes so that their permeability is disturbed which results in cells being unable to carry out life activities so that their growth is inhibited and dies (Arlofa, 2015) [3].

Probiotics are nonpathogenous remains that when consumed in sufficient quantities can provide benefits for the health and physiology of the body (FAO / WHO, 2002) [5]. Probiotics will affect the physiological function of the intestine directly or indirectly by modulating the intestinal microflora and mucosal immune system, especially the gastrointestinal mucosa. According to Surono (2004) [17], lactic acid bacteria produce lactic acid and some metabolite compounds are antimicrobial, such as: hydrogen peroxide ($H_2O_2$), carbon dioxide ($CO_2$), diacetyl and bacteriocins. Lactic acid will lower the pH around the intestinal tract to 4-5, thus inhibiting the growth of putrefactive bacteria and *E. coli* that require an optimum pH of 6-7. The growth of pathogenic bacteria in the intestine is also suppressed by the bactericidal effect of $H_2O_2$ due to the oxidation of bacterial cells, namely the sulfidril group of cell proteins which causes denaturation of a number of enzymes and the occurrence of lipid peroxidation which causes the permeability of cell membranes to increase. $H_2O_2$ also acts as a precursor to the formation of free radicals as bactericide such as superoxide ($O_2^-$) and hydroxyl ($OH^-$) radical compounds that can damage DNA. In addition, the $H_2O_2$ formation reaction will bind oxygen so as to form an anaerobic atmosphere that is uncomfortable for aerobic bacteria. Bacteriocins are peptides that are antibacterial

## Conclusion

Based on the results of this study, it can be concluded that the administration of *jamu* makarens at the level of 2-6% in the ration has not affected the growth of broiler chickens, but can reduce feed consumption and drinking water, FCR values, and suppress the number of pathogenic bacteria (*E. coli, Coliform* and *Salmonella*) in the intestines. The level of 2% in the ration is already effective enough to increase the feed efficiency and suppress the number of pathogenic bacteria (*E. coli, Coliform* and *Salmonella*) in the intestines of broiler chickens.

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## Conflict of interest declaration

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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