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Antibacterial Test of Dadih Against Pathogenic Bacteria and Its Effects on Native Chicken Immunity Organs

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Abstract. Dadih contains lactic acid bacteria that are antimicrobial so that it can be utilized to maintain the health of poultry such as native chickens. This study aims to prove the ability of dadih to suppress the growth of pathogenic bacteria, namely *Staphylococcus aureus*, *Escherichia coli* and *Salmonella*. This study will also try to find out invivo effect of dadih on the organ immunity of native chicken infected with *Salmonella*. Antimicrobial research uses disc diffusion method with antimicrobial zone parameter. The invivo study was conducted using a completely randomized design with 4 treatments and five replications where the parameter was the development of immunity organs namely thymus, bursa fabrisius and spleen. The results showed that the antimicrobial test of dadih against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* caused a zone of inhibition of 7.84, 6.63 and 8.34 mm. Dadih treatment significantly affected the percentage of thymus, bursa fabrisius and spleen. As conclusion that dadih is able to suppress the growth of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* at medium strength with a range of inhibition zone between 6.63-8.34. Dadih is also able to maintain the health of native chicken.

Keywords: antibiotic, bursa fabrisius, dadih, native chicken, spleen, thymus

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1. Introduction

The European Union imposed a ban on the use of antibiotics as feed additives. Antibiotics can also no longer be applied to compensate for growth or poor hygiene. This is due to possible concerns regarding their possible absorption in livestock products. Furthermore, resistance in animals might be transmitted to humans and harm to their health. Exceptions to the use of antibiotics are allowed to treat cases of diseases [10] – [2]. The presence of antibiotic residues in meat can have detrimental effects. In Denmark, previously, they used over 105 metric tonnes of antibiotics for growth promotion but currently the antibiotic use is 0 [3].

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One of the potential alternatives for the development of alternative antibiotics is to use probiotics which are natural materials to boost the immune system of livestock. One example of probiotics is buffalo milk dadih. People in West Sumatra consume dadih which they calls dadih as a side dish and traditional medicine. Dadih is produced from a traditional fermentation process that uses bamboo tubes as containers and banana leaves as covers. The fermentation process can occur due to the presence of microorganisms on the bamboo segment and also on the inner surface of the bamboo tube [4]. Dadih contains a lot of lactic acid bacteria which is about 75%, for example, Lactobacillus and Bifidobacterium [5].

An acidic pH of dadih has the ability to suppress microbial growth. In addition, dadih contains lactic acid bacteria that produce bacteriocin which can inhibit pathogenic bacterial strains such as *Listeria*, *Clostridium*, and *Salmonella*. Bacteriocins are cationic peptides that cause cell death of pathogenic bacteria by forming pores and discharging the cytosolic contents of pathogenic bacterial cells. At the same time, bacteriocins are able to modulate host immunity thereby improving host health [6]. Bacteriocins are antimicrobial peptides produced by lactic acid bacteria that generally suppres or kill pathogenic bacteria in the gut and alter the composition of the gut microbiota in animal models such as mice, pigs, and chickens [7] – [8].

2. Materials and Methods

2.1. Materials

In this study, materials used are buffalo milk, green bamboo with its local name Gombong bamboo (*Gigantochloa verticillata*) for container in dadih making, and dadih. This type of bamboo is most commonly planted in residential neighborhoods by the community. The materials used for in vitro test were dadih, nutrient agar (NA) media, Muller Hinton Agar (MHA) media, distilled water, 70% alcohol, paper disc, *E.coli bacteria*, *Staphylococcus aureus*, and *Salmonellla*. The tools needed are petri dishes and disc diffusion.

2.2. Methods

This study used invitro and invivo tests. The in vitro test was conducted to test the antibacterial power of dadih against pathogenic bacteria while the invivo test was to test the effect of dadih consumption on the health organs of native chickens namely thymus, bursa fabrisius and spleen. Invitro test using disc diffusion method and measurement of inhibition/antibacterial zone. Sterile paper discs were dripped with 20 micron liters of dadih and allowed to stand for approximately 3 hours. Bacterial streaking was performed using a sterile cotton swab on the surface of MHA media with bacterial suspension in 0.5 McFarland turbidity. Dadih-treated paper discs were placed into the agar that had been streaked with bacterial suspension. Incubated for 18-24 hours. The antibacterial activity of the dadih was observed and the diameter of the clear zone formed around the paper disc was measured as antibacterial zone.

In the in vivo study, after the chickens were slaughtered, the organs that reflect the health of the chicken body were carefully taken and weighed, including the spleen, thymus, and bursa fabrisius using analytical scales. Furthermore, each sample was divided by the live weight of the chicken multiplied by 100. Invivo test was conducted on chicken. Reseach design used Non-factorial Completely Randomized Design (CRD) consisting of 4 (four) treatments and 5 (five) replications, where: P0 = Control without dadih administration; P1 = 25% dadih added with 75% aquadest; P2 = 50% dadih added with 50% aquadest; P3 = COLIMAS (antibiotic). The treatment was carried out through administration in drinking water. The research data obtained will be analysed using analysis of variance (ANOVA) and if the results obtained are very real or real then continued by using Duncan's Multiple Range Test.

3. Results and Discussion

3.1. Antibacterial Test

Antimicrobial zone is a parameter of dadih's ability to suppress or kill pathogenic bacteria. Four catagories of the ability to kill bacteria are very strong if the antibacterial zone >20 mm, strong 10-20 mm, medium 5-10 mm and weak <5 mm [9]. [10] found that diameter of zone of inhibition of Lactobacillus ranged from 9 ± 0.0 to 27 ± 2.83 mm. Lactobacillus has the power to inhibit Salmonella. In this study, it was proven that dadih was able to suppress the growth of *Staphylococcuc aureus, Ecscherechia coli* and *Salmonella* at medium category while dadih has the highest antibacterial zone on *Salmonella*. This ability of dadih will benefit the host, in this case the native chicken. Native chickens kept in rural areas and allowed to roam in the environment will be more easily infected with pathogenic bacteria. Therefore, the routine feeding of dadih causes the chickens to have strength against such bacterial infections. Salmonellosis is one of the major bacterial infections affecting commercial poultry, causing losses in poultry production. Fowl typhoid and pullorum disease, which are widespread septicemic diseases, are caused by *S. gallinarum* and *S. pullorum* respectively and infect mainly chickens and turkeys [11]. Salmonellosis is highly detrimental to the livestock industry. But an even worse consequence is that humans who consume chicken meat contaminated with *Salmonella* experience serious health problems [12].

Table 1. Antibacterial Test Result of Dadih on *Staphylococcus aureus, Escherichia coli* and *Salmonella*

Parameter	Antibacterial zone (mm)
Staphylococcus aureus	7.84
Escherichia coli	6.63
Salmonella	8.34

Akanbi et al. stated that E. coli infection causes poultry to exhibit reproductive lesions which correlates with decreased egg production. E. coli also causes colibacillosis in poultry flocks [13].

Avian colibacillosis causes septicaemia (colisepticaemia), pneumonia, yolk-sac infection, omphalitis, cellulitis [14]. S. aureus is one of the most common pathogens found in poultry farms. This bacterium causes asdermatitis, arthritis, osteomyelitis, synovitis, teno-synovitis, femoral head necrosis, bumble-foot, and comphalitis [15]. However, what is not expected is contamination from poultry meat infected with S. aureus that is eaten by humans causing the toxin it produces, namely Enterotoxigenic causing foodborne poisoning and toxic shock syndrome [16] – [17].

3.2. Immune Organ Percentage of Native Chicken

3.2.1. Thymus

Thymus percentage is showed in "Table 2". Feed provides nutrients for the growth and development of primary lymphoid organs, namely thymus, bursa fabricius and and secondary lymphoid organs, namely the spleen [18]. Feed containing probiotics will stimulate the chicken's immune system so that it can control disease. The indication of the effect of this phenomenon is the size/percentage of lymphoid organs such as thymus. Research conducted by [19] found the percentage of thymus between 0.24 to 0.28. This study provides probiotics *Bacillus sp*. Research conducted by [20] with the treatment of giving lactic acid bacteria-based synbiotic rations found the percentage of thymus around 0.102 to 0.128. Research by [21] found the percentage of thymus in the range of 0.22 to 0.24 with the treatment of feeding consist of *Lactobacillus sp*.

Table 2. Thymus Percentage (%) due to Application of Dadih

Treatment	Replications					Total	Average + SD
	1	2	3	4	5		
P0	0.12	0.12	0.13	0.11	0.12	0.60	0.12 ± 0.007a
P1	0.21	0.19	0.19	0.18	0.20	0.95	0.19 ± 0.011ab
P2	0.27	0.26	0.28	0.27	0.27	1.35	0.27 ± 0.007c
P3	0.24	0.22	0.23	0.24	0.22	1.15	0.23 ± 0.010bc

Note: Different superscripts in the same column indicate significant differences ($P < 0.05$).

In this study, dadih feeding was better than the previous studies. This is probably because dadih contains various lactic acid bacteria as probiotics. Dadih in this study is the result of buffalo milk fermentation in a bamboo tube. In buffalo milk itself, there are already some lactic acid bacteria and with the fermentation process of the milk in bamboo tubes, the variety of lactic acid bacteria derived from bamboo canisters increases. This research will be easier to follow by farmers because it will be easier to get a variety of lactic acid bacteria in dadih than to do the process to get isolates.

3.2.2. Bursa Fabrisius

Antibody formation in chickens takes place intensively by B lymphocytes in the bursa Fabricius. The function of the bursa fabricius becomes clearer, namely as the central organ of B cell formation in the avian immune system [22]. Whereas in other species, B cell formation mainly

occurs in the bone marrow. Furthermore, another study reported that removal of the bursa of Fabricius (bursectomy) in chickens significantly reduced the level of immunoglobulins, especially IgG/IgY.

Table 3. Bursa Fabrisius Percentage (%) by Application of Dadih

Treatment	Replications					Total	Average + SD
	1	2	3	4	5		
P0	0.11	0.11	0.11	0.12	0.10	0.55	0.11 ± 0.007a
P1	0.11	0.12	0.12	0.11	0.11	0.55	0.11 ± 0.005a
P2	0.17	0.16	0.16	0.18	0.18	0.85	0.17 ± 0.010ab
P3	0.12	0.16	0.16	0.17	0.15	0.8	0.16 ± 0.019ab

Note: Different superscripts in the same column indicate significant differences ($P < 0.05$).

In this study, it was seen that the percentage of bursa was better with the provision of dadih. The best bursa percentage occurred with 50% dadih and this treatment was equivalent to the positive control, namely the treatment of Colimas antibiotics. Research conducted by [20] with the treatment of lactic acid bacteria-based synbiotic rations found the percentage of bursa around 0.2 to 0.3. Research by [21] found the percentage of bursa in the range of 0.11 to 0.14 with the treatment of *Lactobacillus sp.* This study is better than research result by [21] because in this study dadih was given, which is a collection of various lactic acid bacteria and fungi. [23] stated that probiotics can affect the work of the immune system through antibodies secreted by B lymphocytes and T lymphocytes, resulting in an increase in the relative weight of immune organs. In this study, there was a significant difference between the treatment without dadih and the treatment with dadih.

3.2.3. Spleen

The size of the spleen organ is influenced by the provision of probiotics and feed nutrients. Giving probiotics to the feed there is an increase in antibody production associated with the size of the spleen organ. Feed or nutrients greatly affect the development of lymphoid organs [24]. Research conducted by [19] found % spleen 0.118 to 0.124 where the treatment of this study was the provision of probiotics *Bacillus sp*. In this study, the percentage of spleen was better than [19]. This may be due to a greater variety of lactic acid bacteria causing better spleen development. The percentage of spleen in this study is within the range of spleen size which according to [24] the normal size of broiler spleen ranges from 0.10-0.23% of live weight. The size of spleen giving 50% dadih is close to the maximum size of 0.18%.

Table 4. Spleen Percentage (%) by Application of Dadih

Treatment	Replications					Total	Average + SD
	1	2	3	4	5		
P0	0.13	0.13	0.14	0.12	0.13	0.65	0.13 ± 0.007a
P1	0.13	0.13	0.14	0.12	0.13	0.65	0.13 ± 0.007a
P2	0.17	0.18	0.19	0.18	0.18	0.90	0.18 ± 0.007ab
P3	0.18	0.17	0.16	0.17	0.17	0.85	0.17 ± 0.007ab

Different superscripts in the same column indicate significant differences ($P < 0.05$).

4. Conclusion and Recommendation

Dadih is able to maintain the health of poultry and this is can be found out with the development of chicken health organ indicators namely thymus, bursa fabrisius and spleen even though the chicken was infected by *Salmonella*. In addition, dadih can be proven to be able to suppress the development of *Salmonella* as evidenced by the antimicrobial test which obtained an antimicrobial zone of 8.34. Dadih is still able to suppress the development of *S. aureus* and *E. coli* as evidenced by the formation of an antimicrobial zone. The utilization of dadih will benefit poultry farms while protecting humans from infection with pathogenic bacteria of poultry origin. Given that making dadih is easier than isolating lactic acid bacteria, it would be more efficient to use dadih in poultry farm.

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