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The Effect of Buffalo Milk Whey on Salmonella Populations in Chickens Infected with *Salmonella sp.*

M.K.Arjuna[1], N.Ginting[1] and S.Pakpahan[2]
[1] Animal Science Study Program, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan Medan 20155, Indonesia
[2] BRIN

Abstract. Bacterial is one cause of health decreased on poultry especially chicken. This study aims to determine the effect of giving buffalo milk whey on the number of salmonella populations found in chickens infected with Salmonella sp. The study was conducted at the Animal Production laboratory, Faculty of Agriculture, and the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The design used was a non-factorial completely randomized design (CRD) with 4 treatments with 5 replications. Treatment consisted of P0 (control without whey administration), P1 (25% whey + 75% distilled water), P2 (50% whey + 50% distilled water), and P3 (antibiotic Colimas). Observation parameters were pH, antimicrobial bacterial test and number of Salmonella sp bacterial colonies. The results showed that the treatment had a real effect on pH, antimicrobial bacterial tests and the number of Salmonella sp bacterial colonies. In conclusion that giving buffalo milk whey to native chickens infected with Salmonella sp. with treatment of 50% buffalo whey + 50% distilled water can increase the pH and reduce the population of Salmonella sp. on chicken digestion so that it can replace the use of commercial antibiotics.
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Keywords: antibiotics, antimicrobial test, buffalo milk whey, *salmonella sp,* native chicken
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1. Introduction

Poultry is one of the largest providers of animal protein sources which is at 1.9 million tons and egg production at 2.0 million tons [1]. Native chicken is one of the meat-contributing poultry. The consumption of native chicken meat has increased by 9.23%. This data explains that the public's interest in native meat has increased so it is necessary to pay attention to its health. One of the causes of decreased health in livestock, especially poultry, is the presence of bacterial.

Salmonella sp is one of the bacteria that causes a disease known as Salmonellosis. In addition to increasing mortality, this disease is also a pathogen that can contaminate meat so that the quality of meat decreases and can infect the consumer's body [2]. Generally, farmers use factory-produced antibiotics to reduce the population of bacterial contamination. These antibiotics can cause residues and resistance in microbes in the body of livestock. Giving natural antibiotics needs

*Corresponding author at: **Faculty of Agriculture, University of North Sumatra, Medan, North Sumatra**
E-mail address: nurzainah@usu.ac.id

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to be considered in addition to minimizing residues and contamination, it can also reduce production costs.

Buffalo milk whey is one example of milk processing made from buffalo milk by utilizing the performance of bacteria. Whey contains many lactic acid bacteria with various strains. In addition to lactic acid bacteria, there are also bioactive peptides which is bacteriocin that act as antioxidants and antibacterials. [3] stated that whey plays an important role as an immunomodulator because there are 36 strains of lactic acid bacteria in buffalo milk whey, all of which can act as probiotics. Based on this statement, the author is interested in conducting research to control the population of Salmonella sp in native chickens infected with Salmonella sp by utilizing the liquid part of buffalo milk curd (whey).

2. Materials and Methods

2.1. Place and Time

The study was conducted at the Animal Production laboratory, Faculty of Agriculture, and the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. It was conducted from June to September 2023.

2.2. Materials and Equipment

The materials used in this study were 20 native chickens, buffalo milk whey, commercial feed, *Salmonella sp* bacterial isolates, EMB (*Eosin Methylene Blue*) solid media.

2.3. Research Methods

This study used a non-factorial Completely Randomized Design (CRD) consisting of 4 (four) treatments and 5 (five) replicates, where:

P0 = Control without whey administration

P1 = 25% whey + 75% aquadest

P2 = 50% whey + 50% aquadest

P3 = COLIMAS (antibiotics)

2.5. Data Analysis

The research data obtained will be analyzed using analysis of variance using *Analysis of Variance* (ANOVA) and if the results obtained are very real or real then continued by using Duncan's *Multiple Range Test* with a significant level of 5% to find out the best treatment.

2.6. Degree of acidity (pH) of buffalo milk whey

First prepare 20 samples of whey, each containing 10 mL of whey. pH measurement is done using a pH meter. A sample of 10 mL in a solid state was taken, then the pH meter was rinsed with distilled water before using the electrode. Then the electrode is dried with tissue paper, then

dipped into the sample. The electrode is allowed to move until the position of the constant number. Read the number shown by the pH meter this is the pH value of the sample.

2.7. Bacterial Antimicrobial Test

Test bacteria (Eschericia coli, Staphylococcus aureus) were prepared by scratching the culture on nutrient agar (strik plate), then incubated at 37 ºC for 24 hours. Subsequently, each test bacteria was refreshed into 10 ml of lactose nutrient broth and incubated until reaching an OD of 0.8 at 37 ºC. One milliliter of each test culture (pathogenic bacteria) was instilled into sterile nutrient agar that had been frozen by the spread plate method and with 2 replicates. Next, 2-4 sterile paper discs were placed on each petri dish for antimicrobial testing. On each paper disc, 100 µl of yeast culture in GDP and in a cell-free broth or broth containing inactive cells was dripped, then the Petri dish was incubated at 25ºC for 5 days (120 hours).

Observations were made every 24 hours by measuring the inhibition zone based on the clear area formed around the disc paper, and observations were made 5 times, namely during the incubation period of the yeast isolate 24, 48, 72, 96 and 120 hours. [4] stipulates a minimum inhibition zone area of 1mm, positive 1 (+) when the clear area is between 2-5 mm and strong inhibitory activity (++) when more than 5mm.

2.8. Colony Count of *Salmonella sp* Bacteria

Total bacterial analysis was carried out using the *pour plate* method according to [5,6]. 20 sterile test tubes were prepared according to the number of samples to be tested. The dilution was done by taking 1 gram of chicken digesta, then adding 9 ml of sterile distilled water and then homogenized. This is a dilution of 10^8, from this dilution 1 ml is taken, 9 ml of distilled water is added and then homogenized. This is dilution 10^7.

Dilution was continued until 10^6. From dilutions 10^8 , 10^7 , and 10^6 ,0.1 ml each at each dilution was taken and diluted three times, grew on *salmonella shigella agar* (SSA), incubated at 37ºC for 18-24 hours. After incubation and the growing bacteria will be counted the number of *colonies* formed by using a *colony counter* or counting manually with the inclusion criteria the number of bacteria is 25 - 250 colonies. After the number of bacteria has been counted and falls within the range of 25 - 250 colonies, it will be entered into the formula. According to [6], total microbial colonies can be calculated using the following formula:

Total microbial colonies can be calculated using the following formula:

$$\text{Number}\frac{\text{bactery}}{\text{gram}} = \text{total colony x}\frac{1}{\text{dilution factor}}$$

3. Results and Discussion

3.1. Degree of acidity (pH) of buffalo whey

Data and statistical analysis of pH test followed by Duncan multiple range test (DMRT). The

mean value, standard deviation and notation of duncan multiple range test (DMRT) test of buffalo whey curd pH results are in Table 1.

Table 1. Degree of acidity (pH) of buffalo curd whey

Treatments	Average pH
P0	4,48[d]
P1	4,60[c]
P2	4,84[b]
P3	4,88[a]

Description: Numbers followed by the same notation in the same row and column indicate not significantly different at the 5% level according to the DMRT test.

Potential of hydrogen ion or pH is defined as the logarithm of hydrogen ion concentration expressed in grams per liter of solution. The pH value indicates the acidity of a material. pH measurements can use colorimetric methods or potentiometric methods. The colorimetric method is a way of measuring pH using indicators that can show certain colors at certain pH conditions. While the potentiometric method is by using a potentiometer that works based on the potential difference between two electrodes in a solution. One of the electrodes is a standard electrode while the other is an electrode that is affected by the solution whose pH will be measured. The potential difference between the two electrodes will cause an electric power that can be measured and converted to numbers. Potentiometers that are widely used are pH-meters [7].

The results of the analysis of variance showed that buffalo milk whey showed a very significant difference (P 0.05) to the pH value. The very significant difference between treatments was due to the different concentrations given different concentrations in each treatment so that the pH analysis results showed an increase along with the addition of a higher concentration of starter culture. The increase in pH value is thought to be due to differences in the activity of lactic acid bacteria that convert carbohydrates in milk into other compounds, namely lactic acid.

The more the addition of starter culture will cause the pH of buffalo curd whey to increase. The increase occurred starting from P0, P1, P2 and finally P3. It shows that P0 gives the lowest average result compared to other treatments. This is because P0 is the optimal point in the addition of starter culture so that less lactic acid is formed compared to other treatments.

The pH value is used as an indicator to determine the acidity of food products. The pH value has a fairly close relationship with the total acid value. The relationship between pH value and acidity is inversely proportional. So that the more acid formed from a fermentation process will cause a decrease in pH value. [8] states that the gel begins to form in fermented milk products at a pH value of 4.6. The pH value of 4.6 is the isoelectric point where casein changes its structure to form a coagulant. The isoelectric point is the point when the charge of positive and negative ions is the same. So that at that point casein cannot dissolve in water because the intersection between casein and lactic acid causes a neutralization process so that casein precipitates.

Milk fermentation is influenced by the growth of microorganisms. The growth of microorganisms during the fermentation process is influenced by the substrate used to grow. The nutrient content contained in the substrate is thought to be a factor that affects microbial growth and development.

Nutritional components needed by microbes are carbohydrates, proteins, lipids, minerals and vitamins. It is known in research that the substrate used for microbial growth is limited. Meanwhile, the high concentration of starter culture makes fermentation run faster. So that secondary metabolic products such as alcohol that are not counted as total acid are formed more and more.

[9] reported that in whey there are other microorganisms besides lactic acid bacteria. These microorganisms are molds and yeasts. Yeast is known to have a role in the fermentation process that produces alcohol. So the results of the pH analysis are known to be not only caused by fermentation activity by lactic acid bacteria but influenced by the activity of yeast that converts carbohydrates into alcohol. The higher concentration of starter culture (P2 and P3) is known to produce a greater pH value than the lower concentration of starter culture (P0 and P1).

P3 and P2 are treatments with high fermentation activity by yeast in addition to fermentation activity by lactic acid bacteria. So that the formation of alcohol that is not counted as titratable acid makes the pH value in these treatments higher than in P1 and P0 which produce higher lactic acid so that the pH value of P1 and P0 is low. The higher the concentration of starter culture, the higher the opportunity for yeast to carry out fermentation activities that produce alcohol which will have an impact on the formation of lactic acid by lactic acid bacteria. It is known that the substrate or place of growth in each treatment is under uniform conditions, namely in volume and nutrient content, and the type of milk used in the study.

The average difference produced in each treatment is due to the influence of the addition of starter culture as a starter for making buffalo milk curd in bamboo segments. It has been previously known that starter culture works as a starter that ferments casein in milk into lactic acid and other metabolite compounds. The starter is obtained from buffalo milk fermented in bamboo tubes. In addition, there is also starter from the bamboo tubes itself. The amount of lactic acid formed is related to the pH value of buffalo milk whey. The higher the lactic acid content of buffalo milk whey, the lower the pH of the buffalo milk whey.

The results showed that buffalo milk whey with the addition of starter or starter culture had a pH range of 4.48 to 4.88. [10] reported that the pH value of curd was 4.5. Meanwhile [11], reported that commercial curd Tilatang Kamang Agam Regency and Lembah Gumanti Solok Regency had a pH value of 4.76 to 4.81. Whey with milk from grass-fed buffalo has a pH of 4.3 [12]. The difference in results is caused by differences in the composition of the raw materials used. On the other hand, there is no SNI for whey, making this product have different results in terms of raw materials, location of manufacture and method or method of manufacture. The results of research [11] that commercial whey in 2 areas namely Tilatang Kamang Agam Regency and Lembah Gumanti Solok Regency has a pH value of 4.76 to 4.81. In research [12] that the pH value of whey with milk derived from buffalo fed with rice straw has a pH of 5. Meanwhile, whey with milk derived from buffalo fed with grass has a pH of 4.3. And whey with milk from buffaloes fed grass, rice and coconut milk has a pH of 4.6. [13] reported that the pH of traditionally fermented goat milk whey in ori bamboo tubes had a pH value of 6.47. Whereas in research [14] that cow's

milk whey with a combination of starters has a pH range between 3.99 to 4.07.

Based on the level of acidity, foods are divided into 2 categories. The first category is food that has high acidity (has a pH below 4.6). Second, foods that have low acidity, which has a pH above 4.6 [15]. So that goat's milk whey fermented with the addition of starter origin is included in the category of high acidic food. The range of pH values of goat milk whey in the study ranged from 4.48 to 4.88.

3.2. Bacterial Antimicrobial Test

Inhibition zone testing on solid media was used to determine the antibacterial effect of buffalo whey curd against the test bacteria Sthaphylococcus aureus and Escherichia coli. The results of the analysis can be seen in Table 2.

Table 2. Results of antimicrobial test analysis of buffalo whey bacteria

Treatment	Antibacterial activity (mm) Escherichia coli	Antibacterial activity (mm) Staphylococcus aureus
P0	0^{d}	0^{d}
P1	$7{,}70^{c}$	$6{,}71^{c}$
P2	$8{,}83^{b}$	$7{,}35^{b}$
P3	$9{,}30^{a}$	$8{,}35^{a}$

Description: Numbers followed by the same notation in the same row and column indicate not significantly different at the 5% level according to the DMRT test.

Table 2 shows the range of inhibition zones formed in buffalo whey curd beverage products against Staphylococcus auerus and Escherichia coli respectively are 0 - 22, 30; 0 - 18.35 mm. The zone of inhibition produced by buffalo whey products in this study differed greatly from research [16] for fermented whey products against Staphylococcus auerus FNCC 0047, Bacillus cereus FNCC 0057, Escherichia coli FNCC 0091 8.69 - 12.57; 10.05 - 14.39; 7.91 - 12.89 mm respectively. This is thought to be due to differences in bacterial species and differences in constituent components.

The higher the treatment concentration, the greater the clear zone area. The clear zone formed is influenced by lactic acid bacteria that produce lactic acid, other organic acids, hydrogen peroxide, and diacetyl and other compounds that are antimicrobial [17]. The results of the analysis of variance showed that the addition of various levels had an effect ($P>0.05$) on antibacterial activity against Escherichia coli bacteria in buffalo whey.

Further test results (“Table 1”) showed that the inhibition zone produced by whey curd with treatment P0 was smaller than treatment P1, P2 and P3 ($P<0.01$). The zone of inhibition increases along with the treatment of buffalo whey (Table 1), the difference in treatment is utilized by Lactobacillus acidophilus bacteria, the accumulation of lactic acid causes a decrease in pH. High lactic acid and low pH have a function as antibacterial, which inhibits the growth of pathogenic bacteria. Escherichia coli was used as an inhibitory effect because it is a pathogenic bacterium that grows optimally at pH 6-7 [18].

According to [19] the criteria for antibacterial power strength are as follows: inhibition zone diameter of 5 mm or less is categorized as weak, inhibition zone of 5-10 mm is categorized as moderate, inhibition zone of 10-20 mm is categorized as strong and inhibition zone of 20 mm or more is categorized as very strong. Based on these criteria, the antibacterial power of buffalo whey on Escherichia coli and Staphylococcus bacteria is included in the moderate inhibition zone category, exclude P0.

The antibacterial activity of buffalo whey beverage products with the addition of Staphylococcus aureus treatment can be seen from the diameter of the inhibition zone. The inhibition zone increases along with the treatment of buffalo whey curd (Table 1). Anova results showed that buffalo whey curd with various treatments affected the inhibition zone of *Staphylococcus aureus*. Further test results (Table 1) showed that the inhibition zone produced by buffalo whey curd with sucrose level treatment P0 was smaller than P1, P2 and P3 treatments ($P<0.05$).

The undissociated lactic acid freely penetrates the cell membrane and then enters the high pH cytoplasm. At high pH conditions (in the cytoplasm), lactic acid dissociates to produce protons that tend to lower the pH of the cytoplasm. The cell will try to maintain its internal pH by neutralizing or forcing out protons. This effort will slow down bacterial growth because growth energy is used to release protons. If the external pH is low and the extracellular acid concentration is high, the load from the cell will be large and the cytoplasmic pH will drop. This is impossible to pass under growth conditions and if it happens, the cell will die. The inhibition of ntibacterial compounds was higher in Staphylococcus aureus compared to Escherichia coli.

3.3. *Salmonella sp*

The identification results of Salmonella Sp. in the small intestine of broiler chickens infected with Salmonella Sp. using SSA (Salmonella Shigella Agar) media. This is in accordance with research [20] that on SSA media macroscopically Salmonella Sp. colonies are characterised by the presence of black spot centre because Salmonella is able to produce H2S. [21] added that on SSA media Salmonella Sp. characterised by the presence of white colonies with black dots

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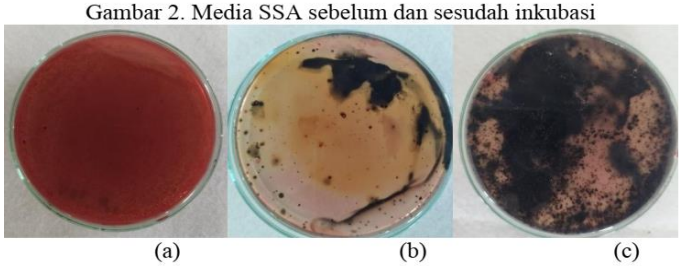
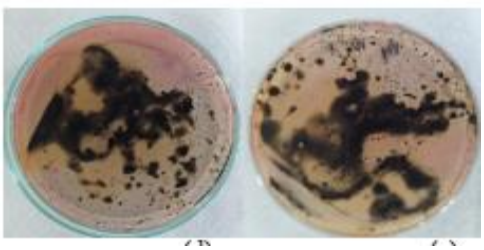
Gambar 2. Media SSA sebelum dan sesudah inkubasi



(a) (b) (c)



Description: (a) SSA media before incubation, (b) P0 after incubation, (c) P1 after incubation, (d) P2 after

incubation, (e) P3 after incubation.

The results of this study also showed the presence of black spots on the SSA media because Salmonella can break down amino acids. SSA media because Salmonella can break down amino acids. The presence of white colour accompanied by black dots on the media because Salmonella sp. breaks down amino acids containing sulfur to form FeS precipitates. FeS salts where the FeS precipitate forms black dots on SSA media [21].

Table 3. *Salmonella Sp.* population (log CFU/ml)

Treatments	Colony average
P0	1,48[c]
P1	3,36[b]
P2	1,34[d]
P3	3,60a

Description: Numbers followed by the same notation in the same row and column indicate not significantly different at the 5% level according to the DMRT test.

"Table 3" shows the Salmonella population on day 30 in broilers with buffalo whey ranged from 1.34-3.60 log CFU/ml. Consecutive population values from lowest to highest were obtained in P3 at 3.6 log CFU/ml, P2 at 1.34 log CFU/ml, P1 at 3.36 log CFU/ml and P3 at 1.48 log CFU/ml. The results of analysis of variance showed that the treatment of infection without antibiotics gave a significant effect (P<0.005) between P0, P1, P2 and P3. Based on the results of the Duncan test, the highest population in the treatment of infection without antibiotics was 3.60 log CFU/ml while the lowest population in the buffalo curd treatment was 1.34 log CFU/ml. Based on this study, it is known that there is no significant difference between the P2 and P3 treatments and they can compensate for P4 which is the use of commercial antibiotics. This is because buffalo whey can inhibit bacterial growth as it contains of bacteriocin [22].

4. Conclusion

4.1. Conclusion

Giving buffalo whey to native chickens infected with *Salmonella Sp.* with treatment 50% buffalo whey + 50% aquadest can increase pH, bacterial antimicrobial test and reduce *Salmonella Sp*. in the digestion of native chickens so that it can replace the use of antibiotics.

4.2. Suggestion

Whey from fermented buffalo milk should be widely utilised in native chicken farming as it can be an alternative antibiotic.

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