# The Ability of *Lactobacillus plantarum* to Reduce the Growth of Bacteria in Beef Meat by the Differences in Temperature and Storage Time in Term of pH and Microbiological Tests

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**ABSTRACT**
Food safety is an effort made to prevent food from contamination or contamination of biological, chemical, and other objects that interfere and harm public health. Foodstuffs derived from livestock are one of the food ingredients that are not durable or easily damaged (perishable). One of the livestock products that are damaged and susceptible to pathogenic microorganisms and spoilage is meat. The quality of the meat can be seen from the pH and the level of microbial contamination in the meat. Meat which generally comes from Slaughterhouses (RPH) has the potential to be contaminated with bacteria immediately after being cut and is marketed to consumers' hands. Butchers generally market meat traditionally so that contamination by bacteria can easily occur. Therefore, it is necessary to control the spoilage bacteria so that the meat has a long shelf life.One alternative for meat preservation that is safe to use is soaking meat using bacteriocin produced by lactic acid bacteria (LAB). One of the *Lactobacillus* genera that has the potential to produce bacteriocins is *Lactobacillus plantarum*. *Lactobacillus plantarum* can the ability to produce lactic acid and lower the pH of the substrate which can inhibit microbial contamination. This study used a factorial completely randomized design (CRD) consisting of 3 factors and 3 replications. The treatments used were *Lactobacillus plantarum* addition factor, temperature and storage time. The results showed that the addition of the percentage of *Lactobacillus plantarum* bacteria in beef with different temperatures and storage periods gave a very significant effect on the pH. Meanwhile, the addition of the percentage of *Lactobacillus plantarum* bacteria in beef with different temperatures and storage periods did not significantly affect the microbiological test.

***Keywords:*** *meat, Lactobacillus plantarum, temperature, storage time, pH, TPC.*

## 1. INTRODUCTION

One of the livestock products that are damaged and susceptible to pathogenic microorganisms and spoilage is meat. One of the livestock products whose needs are currently increasing among the community is meat. Meat is a livestock product that is easily contaminated by pathogenic bacteria because of its high protein content and complete balance of essential amino acids. Beef is easily damaged if not handled properly, then the meat will undergo physical, chemical, and microbiological changes [1].

The quality of the meat can be seen from the pH value and the level of microbial contamination in the meat. Microbial contamination in meat is caused by microorganisms *Escherichia coli*, *Salmonella sp* and *Streptococcus aureus* that can grow on meat [2]. Protein degradation is caused by the occurrence of pathogenic microbial contamination in meat which causes damage to cells in the meat so that the meat easily becomes rotten. Meat that generally comes from Slaughterhouses (RPH) has the potential to be contaminated with

 

bacteria immediately after being cut and is marketed to consumers' hands.

The content of bacteria in meat that exceeds SNI standards, then the meat is not suitable as food because it can cause damage and can cause disease. Therefore, it is necessary to control the spoilage bacteria so that the meat has a long shelf life. One alternative for meat preservation that is safe to use is soaking meat using bacteriocin produced by lactic acid bacteria (LAB). One of the *Lactobacillus* genera that has the potential to produce bacteriocins is *Lactobacillus plantarum*. *Lactobacillus plantarum* can inhibit pathogenic microorganisms greater than other LAB [3]. In addition, *Lactobacillus plantarum* can produce lactic acid which can reduce the pH of the substrate which can inhibit contamination of pathogenic microorganisms. However, research on the use of *Lactobacillus plantarum* on fresh beef has never been done, but on processed beef products it has been done. Therefore, research on the ability of *Lactobacillus plantarum* in suppressing bacterial growth in beef with different temperatures and storage periods for pH and microbiological tests needs to be done to answer the challenges mentioned above.

## 2. MATERIALS AND METHODS

### *2.1. Place and time of research*

This research was conducted at the Laboratory of Meat Processing Science and Technology, Animal Husbandry Study Program, Faculty of Agriculture, Syiah Kuala University, and the Laboratory of Milk Processing Technology, Animal Husbandry Study Program, Faculty of Agriculture, Syiah Kuala University, Banda Aceh. This research was conducted on 16 September-17 December 2020.

### *2.2. Research tools and materials*

The tools used in this study consisted of pots, stirrups, pans, knives, cutting boards, containers, sample cups, sample plastics, label paper, masks, gloves, rags, blenders, incubators, *ose*, autoclaves, Erlenmeyer, cups. Petri, sterile, measuring cup, hot plate, digital scale, bunsen, pH meter, duct tape, cotton, tissue, aluminum foil, sample bottle, test tube, diluent tube, and stationery. The materials used in this study were beef, tomato juice, 70% alcohol, Aquades, NA, MRSB, brown sugar and *Lactobacillus plantarum* bacteria.

### *2.3. Research procedure*

The room to be used is cleaned first, such as the floor and table using floor cleaner and alcohol. The tools used for research are cleaned and sterilized before use by spraying 70% alcohol on the tools to be used. 1 ml of *Lactobacillus plantarum* culture was taken and then the culture was inoculated on 9 ml of de Man Rogosa Sharp Broth (MRSB) media. After that, the culture was incubated at 37$^{o}$C. The *Lactobacillus plantarum* bacteria in this study were ordered from the Microbiology Laboratory of the Center for Food and Nutrition Studies, Gajah Mada University (UGM), Yogyakarta. The culture of *Lactobacillus plantarum* obtained was in the form of isolates in de Man Ragosa Sharpe Broth (MRSB) agar medium. Culture propagation was carried out by inoculating culture stock into MRSB liquid media to which 20% tomato extract had been added and incubated at 37°C for 24 hours [4].

The working culture of *Lactobacillus plantarum* was carried out by adding 6% of *Lactobacillus plantarum* bacteria to 250 ml of meat broth to which 50 g of the brown sugar had been added. Then the working culture was incubated for 24 hours. The beef sample used was beef from the thigh of 500 g which was obtained from the Ulee Kareng market. The prepared beef was then cut and weighed at 10 g/sample each. After the cutting process, the sample is separated and placed in a sample container. The meat that had been placed in the sample container was then soaked using a working culture of *Lactobacillus plantarum* as much as 0% (control) and 6%. The pH test was carried out on the sample using a pH meter and the TPC test was carried out using the colony counter pour plate method on NA agar media.

### *2.4. Statistical analysis*

The analysis was carried out using analysis of variance (ANOVA) of variance. If there is a difference between the treatments, the Duncan Multiple Range Test (DMRT) will be carried out [5].

## 3. RESULTS AND DISCUSSION

### *3.1. Potential Hydrogen (pH)*

The Potential of hydrogen (pH) is a measure that describes the degree of acidity or alkalinity of a solution, pH is measured on a scale of 0-14 [6]. The pH value of a solution is neutral if it is at 7, acidic if it is less than 7, and alkaline or alkaline if the pH is more than 7. beef with different temperatures and storage times can be seen in Table 1.

The results of the analysis of variance showed that the addition of *Lactobacillus plantarum* (a) with storage temperature (b) and storage time (c) on the pH value of beef had a very significant effect ($P<0.01$) and there was an interaction between the three treatment factors on the pH of the meat.

Based on the results of Duncan's further test analysis showed that there was a difference between treatment A1 (0%) with storage temperature B1 (28$^{0}$C) and

**Table 1.** Potential hydrogen of beef

| Addition *L. plantantarum* | Temperature | Storage Time | | Average ab | Average a |
|---|---|---|---|---|---|
| | | c1 (0 Day) | c2 (1 day) | | |
| a1 (0%) | b1 (28$^0$C) | 5.69±0.12$^c$ | 6.23±0.13$^a$ | 5.96$^a$ | 5.92$^a$ |
| | b2 (10$^0$C) | 5.91±0.09$^b$ | 5.84±0.06$^{bc}$ | 5.87$^a$ | |
| a2 (6%) | b1 (28$^0$C) | 5.40±0.04$^d$ | 5.02±0.20$^e$ | 5.21$^b$ | 5.33$^b$ |
| | b2 (10$^0$C) | 5.48±0.09$^d$ | 5.43±0.05$^d$ | 5.45$^b$ | |
| Average ac | | 5.80$^a$ | 6.03$^a$ | | |
| | | 5.44$^b$ | 5.22$^b$ | | |

Note: different $^{abc}$ superscripts in the same row or column show a very significant difference (P<0.01)

**Table 2.** TPC test values for beef.

| Addition *L. plantantarum* | Temperature | Storage Time | | Average ab | Average a |
|---|---|---|---|---|---|
| | | c1 (0 Day) | c2 (1 day) | | |
| a1 (0%) | b1 (28$^0$C) | 8.09±0.37 | 9.00±0.01 | 8.55 | 8.17$^a$ |
| | b2 (10$^0$C) | 7.46±0.45 | 8.14±0.23 | 7.80 | |
| a2 (6%) | b1 (28$^0$C) | 7.50±0.16 | 7.76±0.65 | 7.63 | 7.68$^b$ |
| | b2 (10$^0$C) | 7.68±0.07 | 7.79±0.65 | 7.74 | |
| Average c | | 7.68$^a$ | 8.17$^b$ | | |

Note: different abc superscripts in the same row or column show a very significant difference (P<0.01)

storage time C2 (1 day) against treatment A2 (6%) with storage temperature B1 (28$^O$C) and storage time C2 (1 day). Table 1shows that the a1b1c2 treatment produces the highest pH with a value of 6.23±0.13$^a$ and the a2b1c2 treatment produces the lowest pH of 5.02±0.20$^e$. The resulting pH indicates that the addition of the percentage of *Lactobacillus platarum* with the same temperature and storage time has a different effect on the pH of the meat. This is presumably because the higher the percentage of *Lactobacillus plantarum* given, the lower the pH produced in the meat. Furthermore, this condition is supported by the presence of lactic acid produced by *Lactobacillus plantarum* which causes a decrease in the pH of the substrate. thus creating an acidic atmosphere. According to [7] the pH of beef ranged from 5.46-6.29.This is in line with research [8] which stated that the addition of a 6% starter level of *Lactobacilus plantarum* to fermented iris jerky had a lower pH than the addition of 3% *lactobacillus plantarum* starter with an average value of 5.21.Table 1 shows that in the treatment a2b1c1 and a2b2c1 there was no significant difference in the pH of the meat. The resulting values for each treatment were 5.40±0.04$^d$ and 5.48±0.09$^d$.This is presumably because the treatment *Lactobacillus plantarum* has not worked optimally both at storage temperatures b1 (28$^0$C) and b2 (10$^0$C) with a storage time of c1 (0 days). Likewise, the a2b2c2 treatment which shows the resulting pH is also not much different, namely 5.43±0.05$^d$. The a1b2c2 treatment showed that the resulting value was not much different from the a1b1c1 and a1b2c1 treatments with an average value of 5.84±0.06b$^c$, 5.69±0.12$^c$, and 5.91±0.09$^b$, respectively. According to [9], stated that the pH of meat will decrease according to storage time. The longer the storage treatment on beef, the lower the final pH of the beef.

### *3.2. Total Plate Count (TPC) Test*

TPC is a calculation method used to calculate the number of microbes contained in samples of food and processed animal products with established standards. The data from the calculation of the average TPC on the ability of *Lactobacillus plantarum* in suppressing bacterial growth in beef with different temperatures and storage periods can be seen in Table 2. The results of the analysis of variance showed that the addition of *Lactobacillus plantarum* (a) with storage temperature (b) and storage time (c) on the TPC value of beef had no significant effect (P>0.05) and there was no interaction between the three treatment factors on the pH of beef.

Based on table 2, it can be explained that the lowest TPC value was found in the addition of *Lactobacillus plantarum* a1 (0%) with a storage temperature of b2 (10$^0$C) and a storage time of c1 (0 days) with an average value of 7.46±0.45. The highest TPC value was found in the addition of *Lactobacillus plantarum* a1 (0%) with a storage temperature of b1 (28$^0$C) and a storage time of c2 (1 day) with an average value of 9.00±0.01. In this study, the addition of the percentage of *Lactobacillus plantarum* in beef with different temperatures and storage periods had no effect, presumably because

during the TPC test the media used was NA media. NA media is a non-selective medium so that when calculating the TPC all bacteria in the petri dish media are included including *Lactobacillus plantarum* bacteria.

The results of the analysis of variance showed that there was a difference between the treatments of *Lactobacillus plantarum* a1 (0%) and a2 (6%). Duncan's further test in Table 2 shows that the TPC value in the treatment of Lactobacillus plantarum a1 (0%) was $8.17^{a}$ higher than that of b2 (6%) $7.68^{b}$. The addition of the percentage of *Lactobacillus plantarum* gave different results, presumably because the addition of the percentage of *Lactobacillus plantarum* could suppress the growth of microorganisms in meat. This is supported by the statement [10] which states that *Lactobacillus plantarum* can inhibit microorganisms because of its ability to produce lactic acid and bacteriocin which can lower the pH of the substrate. Likewise, with the storage time of c1 (0 days) and c2 (1 day), there was a difference between the two treatment factors on the TPC value. This is because in c2 storage (1 day) *Lactobacillus plantarum* can work well. [11] stated that bacteriocin was able to work effectively at a room temperature of $27^{0}$C.

Based on the results of the analysis of variance, there was an interaction between the treatment of *lactobacillus plantarum* (a) and storage temperature (b). However, based on Duncan's further test, it did not show a significant difference in the value of the reaction between the treatment factors in Table 2.

## 4. CONCLUSION

Based on the results of the research conducted, it can be concluded that the addition of the percentage of starter *Lactobacillus plantarum* with different temperatures and storage periods can reduce the pH of beef and there is an interaction between the three treatment factors. While the addition of *Lactobacillus plantarum* percentage treatment with different temperatures and storage time did not significantly affect the microbiological test and there was no interaction between the three treatment factors.

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