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Use of Lactobacillus Plantarum Culture and Glycerol in Beef Stored at Low Temperature

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A B S T R A C T

This study aims to determine the extent of the effect of the use of L. plantarum and glycerol on the quality and shelf life of beef. The study used 2,400 grams of fresh beef foreleg and divided into 48 pieces with each piece weighing ± 50 grams. The preservatives used were L. plantarum and glycerol. The study was conducted through an experiment using a Completely Randomized Design with a split plot in time pattern (Steel and Torrie, 1994). The treatment formulation was arranged as follows: Factor A (main treatment) is L. plantarum culture plus glycerol A1: 10% L. plantarum culture and 0% glycerol. A2: 10% L. plantarum culture and 5% glycerol, A3: 10% L. plantarum culture and 10% glycerol, A4: 10% L. plantarum culture and 15% glycerol. Factor B (sub treatment) is the storage time of meat at low temperature (4 °C) where B1: Storage time 4 days, B2: Storage time 8 days, B3: Storage time 12 days, B4: Storage time 16 days. The variables observed in this study were Water Content, pH, and Number of Lactic Acid Bacteria Colonies. The results of the diversity analysis showed that the use of L. plantarum culture and glycerol in beef had a very significant effect ($P < 0.01$) on the water content of the meat, pH and the number of lactic acid bacteria colonies. However, the duration of storage at low temperatures had a non-significant effect ($P > 0.05$) on water content. While for pH and the number of lactic acid bacteria colonies were very significant ($P < 0.01$)

INTRODUCTION

Meat is one of the food ingredients of animal origin that has enormous benefits for humans, because it contains protein composed of essential amino acids, is easily digested and is a source of vitamins, fats and minerals. Beef is a perishable animal product that is easily contaminated with microorganisms. The damage that occurs can be in the form of physical, chemical and microbiological damage which results in changes in meat quality (Priyanto et al. 2015).

Preservation is a human action carried out on foodstuffs with the aim of inhibiting meat deterioration and maintaining meat quality. Storage at low temperatures is one way of preservation to slow down the speed of development of microorganisms. But preservation with low temperatures alone does not necessarily extend the shelf life, because there are still groups of psychrophilic microorganisms including pathogens that can develop (Dasir and Yani, 2020).

Therefore, it is necessary to modify low-temperature storage in other ways, so that the shelf life of meat at low temperatures is extended with guaranteed safety. One of the ways that can be done to maintain the quality and at least suppress the occurrence of meat damage stored at low temperatures is by using lactic acid bacteria (Lactobacillus plantarum) and glycerol. The application of lactic acid bacteria as a preservative in meat stored at low temperatures has often been done, but the combination of lactic acid bacteria with glycerol has not been explained. Lactobacillus plantarum is a type of lactic acid bacteria that at low pH can produce large amounts of lactic acid. By decreasing the pH value, it can inhibit the growth of destructive and pathogenic bacteria in meat. In addition to lactic acid, L. plantarum can also produce a number of antimicrobial compounds such as propionic acid, hydrogen peroxide (H2O2) and bacteriocins (Wihartati et al., 2022)

LITERATURE REVIEW

In the cell wall of L. plantarum there is glycerol so that the addition of glycerol can further activate the bacterial cells which function as preservatives. In addition, the use of glycerol as much as 20% is also used for storage of lactic acid bacteria cultures at freezing temperatures. So that by combining L. plantarum culture with glycerol, it is expected to activate and maintain the bacterial culture in a material. The mechanism of glycerol preservation is by absorbing food water through the ionization process, because glycerol has OH ions that can bind food water when glycerol breaks down with food water. Apart from being a preservative, glycerol also has advantages, including not changing many physical properties, can add a little sweetness, is not toxic or produces toxins, prevents discoloration and softens the product (Wardana et al., 2019). Based on the above background, research was conducted through experiments using L. plantarum culture and glycerol on beef stored at low temperature (4°C).

METHODOLOGY

1. Research Materials

The materials used in this study were fresh beef quadriceps as much as 2400 grams purchased at the Tikala Manado Slaughterhouse (RPH), glycerol & L. plantarum culture obtained from the Microbiology Laboratory PAU Food and Nutrition Gadjah Mada University Yogyakarta. Chemicals used were distilled water, pH 4 and pH 7 buffer solutions, MRS (Man Rogosa and Sharpe) Broth and MRS Agar media, 70% alcohol, peptone water. The equipment used were refrigerator, incubator, oven, autoclave, desiccator, analytical balance, pH meter, erlenmeyer, colony counter, blender, goblet, watch glass, petri dish, test tube, plastic bag, cutting board, knife, pipette, aluminum foil, cotton.

2. Research Design

This research was conducted through an experiment using a completely randomized design, with a split plot in time pattern (Steel and Torrie, 1994 in Muhajirin et al., 2024). The treatments in this experiment consisted of: Factor A (main treatment) is the culture of L. plantarum bacteria plus glycerol which is arranged as follows: A1 : 10% L. plantarum culture and 0% glycerol, A2: Culture of L. plantarum 10% and glycerol 5% of the sample weight. A3 : L. plantarum culture 10% and glycerol 10%, A4: L. plantarum culture 10% and glycerol 15%. Factor B (sub treatment) was the length of meat storage at low temperature (4°C) which was arranged as follows: B1 : 4 days of storage, B2: 8 days of storage, B3: 12 days of storage, B4: 16 days of storage.

3. Research Variables

The variables observed in this study were moisture content, pH, number of lactic acid bacteria colonies.

Data Analysis

Data from observations were tabulated and analyzed by analysis of variance, and if there were differences between treatments, it was followed by a further test of honest real difference (BNJ).

4. Research Procedure

Starter Culture Preparation Procedure

a. 500g tomatoes were washed and cut into 2 pieces and placed in a watch glass.
b. Sterilized for 20 minutes at 121°C.
c. MRS media was weighed as much as 6.2 g, put in an erlenmeyer then mixed with 20 ml tomato extract and 80 ml distilled water then stirred.
d. Transferred into 20 test tubes using a pipette as much as 5 ml per tube.
e. Sterilized for 20 minutes at 121°C.
f. L. plantarum cultures were grown on sterilized MRS media.
g. Incubated for 48 hours at 36°C.

Treatment

a. Meat was first cleaned and washed, then drained.
b. The meat was weighed as much as 2400 g and cut into 48 thinly sliced parts with a thickness of 2 cm, a length of 10 cm and a weight of ± 50 g each.

c. L. plantarum culture and glycerol were prepared with a percentage of 0%, 5%, 10%, and 15% of the sample weight, respectively.

d. The meat slices were mixed with 10% L. plantarum culture and the predetermined percentage of glycerol (0%, 5%, 10%, and 15%, respectively).

e. After that, the meat slices were placed in plastic bags and stored at a low temperature of 4°C or as long as the treatment was determined.

f. The moisture content, pH and number of bacterial colonies of beef were analyzed with an interval of 4 days until the 16th day.

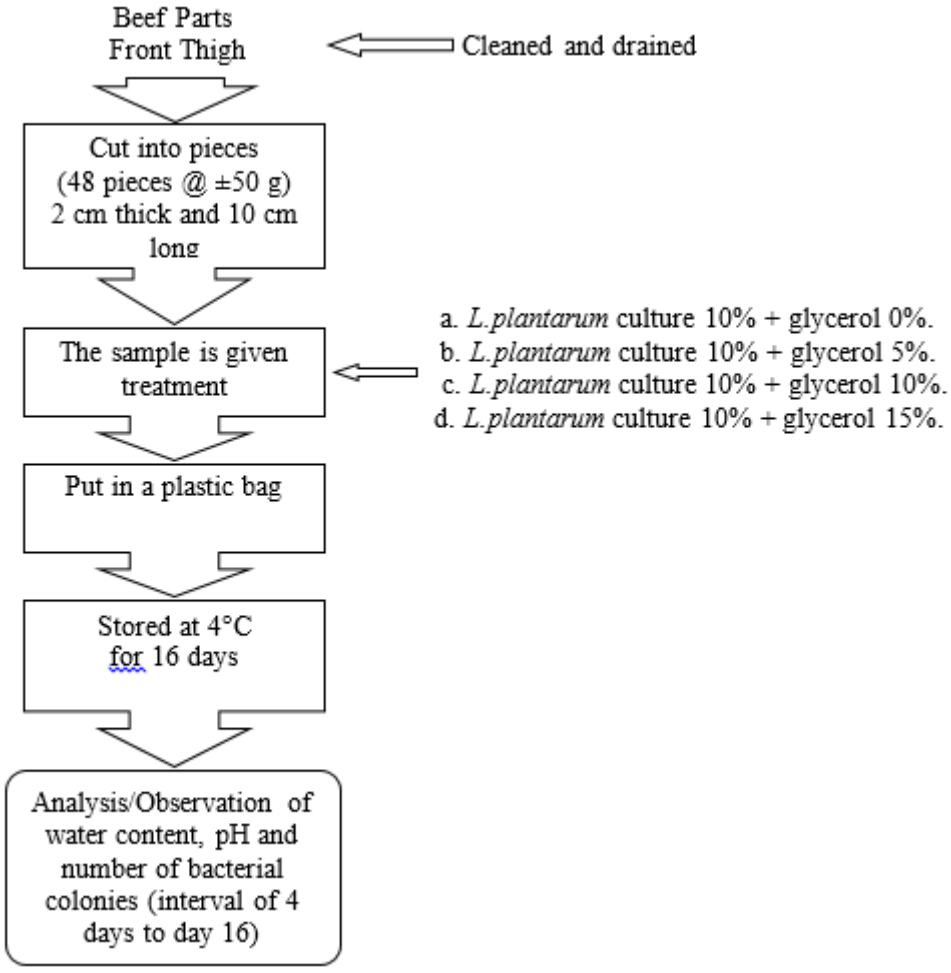

Figure 1. Flowchart of Research Procedure

RESULTS AND DISCUSSION

Beef Moisture Content

The observation data for the effect of using Lactobacillus plantarum culture and glycerol on the average beef moisture content after being stored at low temperature (4°C) for 4 to 16 days are listed in Table 1.

Table 1. Average Beef Moisture Content After Feeding L. Plantarum and Glycerol and Stored at Low Temperature (4°C) for 4 Days, 8 Days, 12 Days, and 16 Days

***L. plantarum* culture and glycerol (A)**	**Length of Storage Days (B)**				**Average**
	4 (B_1)	**8 (B_2)**	**12 (B_3)**	**16 (B_4)**	
A_1 (10% + 0%)	79,40	79,22	79,38	79,48	**79,37**
A_2 (10% + 5%)	79,15	78,37	79,07	79,38	**78,74**
A_3 (10% + 10%)	78,17	78,10	78,13	78,16	**78,11**
A_4 (10% + 15%)	74,76	76,73	75,88	76,86	**76,06**
Average	**77,87**	**78,10**	**78,12**	**78,22**	**78,08**

The addition of 5 to 15% glycerol in 10% L. plantarum culture used in beef caused a decrease in the average water content from 79.37 to 76.06. While the meat storage duration of 4 to 16 days at low temperature caused an increase in the average moisture content from 77.87 - 78.22. he results of the analysis of variance showed that the use of L. plantarum culture and glycerol in beef had a significantly different effect ($P < 0.01$) on meat moisture content. With the understanding that the addition of glycerol at the level of 5 to 15% in the 10% L. plantarum culture used in beef, statistically caused differences in the moisture content of the meat. Further analysis by orthogonal polynomial comparison showed that the addition of glycerol at levels of 5 to 15% in L. plantarum 10% culture had a very significant effect ($P < 0.01$) on reducing beef moisture content.

The decrease in meat moisture content was proven mathematically to follow the equation = 78,8706 – 0,1054x or more clearly can be seen in Figure 2.

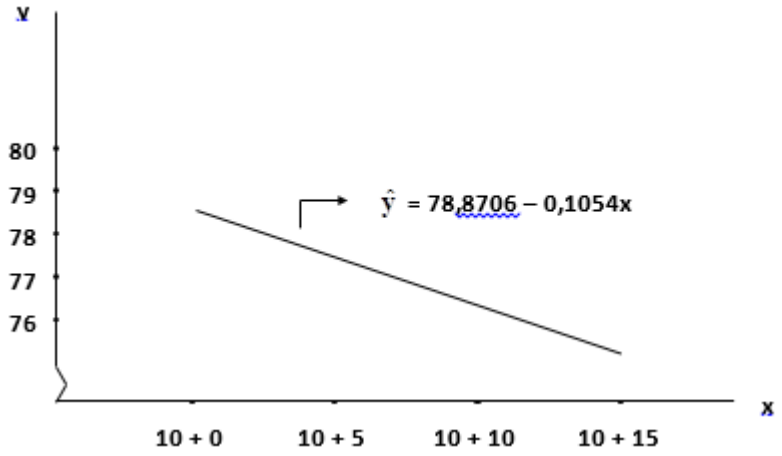

Figure 2. Relationship Graph Between 10% L. Plantarum Culture and 0 To 15% Glycerol (X) And Beef Moisture Content (Y)

The decrease in beef moisture content (Figure 2) shows that glycerol has hygroscopic properties and therefore the higher the level of glycerol in L. plantarum culture used in meat, the lower the moisture content of the meat. According to Rotinsulu et al. (2024), glycerol is one type of humectant with the

main properties of water-soluble and hygroscopic/can bind water. Therefore, according to Wardana et al. (2019) that the mechanism of glycerol preservation is to absorb food water through the ionization process, because glycerol has OH ions that can bind food water when glycerol breaks down with food water. The variances of factor B and factor AB were not significantly different, indicating that the moisture content of beef using L. plantarum culture and glycerol stored at low temperature was the same at 4 days, 8 days, 12 days, and 16 days of storage. This means that the effect of adding glycerol at levels of 5 to 15% in 10% L. plantarum culture on meat moisture content was not affected by the length of storage at low temperatures from 4 to 16 days.

Beef pH

The observation data for the effect of using L. plantarum culture and glycerol on the average pH value of beef after being stored at low temperature (4°C) for 4 to 16 days are listed in Table 2.

Table 2. Mean pH Value of Beef After Feeding L. Plantarum and Glycerol and Stored at Low Temperature (4°C) for 4 Days, 8 Days, 12 Days, and 16 Days.

***L. plantarum* culture and glycerol (A)**	**Length of Storage Days (B)**				**Average**
	4 (B_1)	**8 (B_2)**	**12 (B_3)**	**16 (B_4)**	
A_1 (10% + 0%)	5,37	5,47	5,48	5,51	**5,46**
A_2 (10% + 5%)	5,32	5,42	5,42	5,57	**5,42**
A_3 (10% + 10%)	5,32	5,33	5,43	5,48	**5,39**
A_4 (10% + 15%)	5,19	5,24	5,25	5,38	**5,27**
Average	**5,30**	**5,37**	**5,40**	**5,47**	**5,38**

The average pH value of meat in this study (Table 2), for factor A, decreased with increasing levels of glycerol 5 to 15% in 10% L. plantarum culture used in beef. Mean while, for factor B, it increased with increasing length of meat storage from 4 to 16 days at low temperature. However, the average pH value of beef is still within the range of fresh meat pH values, as stated by Soeparno (2007); Silvia et al. (2022) that the pH value of meat after slaughter ranges from 5.1 - 6.1.

The results of the analysis of variance showed that the use of L. plantarum culture and glycerol in beef and the length of storage at low temperatures had a significantly different effect ($P < 0.01$) on the pH value of meat, while the variety of AB factors had a significantly different effect ($P > 0.05$) on the pH value of meat.

Further analysis with orthogonal polynomial comparisons for factor A, showed that the addition of glycerol at the level of 5 to 15% in L. plantarum 10% culture had a very significant effect ($P < 0.01$) on reducing the pH value of the meat as proven mathematically following the equation = 5,4287 – 0,0060x or more clearly can be seen in Figure 3.

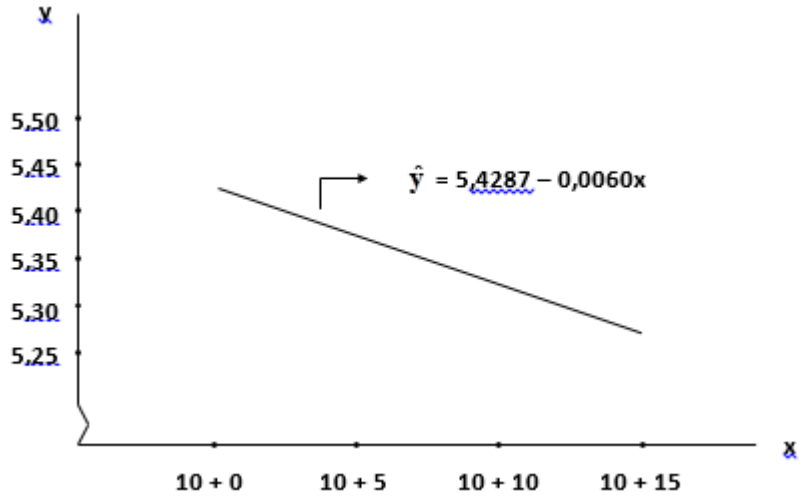

Figure 3. Relationship Graph Between the Use of 10% L. Plantarum Culture and 0 To 15% Glycerol (X) and the Average Ph Value of Beef (Y)

The decrease in the pH value of meat in Figure 3 indicates an increase in lactic acid production in meat due to the addition of glycerol at levels of 5 to 15% in 10% L. plantarum culture. This means that the addition of 5 to 15% glycerol in L. plantarum culture can increase the activity of L. plantarum bacteria to produce lactic acid, causing a decrease in the pH value of meat. According to Solehah (2022); Maria et al. (2024) lactic acid bacteria are bacteria that produce lactic acid, and the lactic acid produced will reduce the pH value of the growth environment and cause a sour taste. In addition, glycerol is hygroscopic (can bind water) so that with the use of glycerol at levels of 5 to 15%, the water content can decrease (Figure 2), which means that the acidity of the meat increases or the pH value of the meat decreases. According to Rotinsulu et al. (2024); glycerol has the main properties of being easily soluble in water and hygroscopic (can bind water).

The results of orthogonal polynomial comparison analysis showed that the length of meat storage at low temperature had a very significant effect ($P < 0.01$) on the pH value of meat. This indicates a functional relationship between storage duration and meat pH value. The relationship mathematically follows the equation = 5,3163 + 0,0067x or more clearly can be seen in Figure 4.

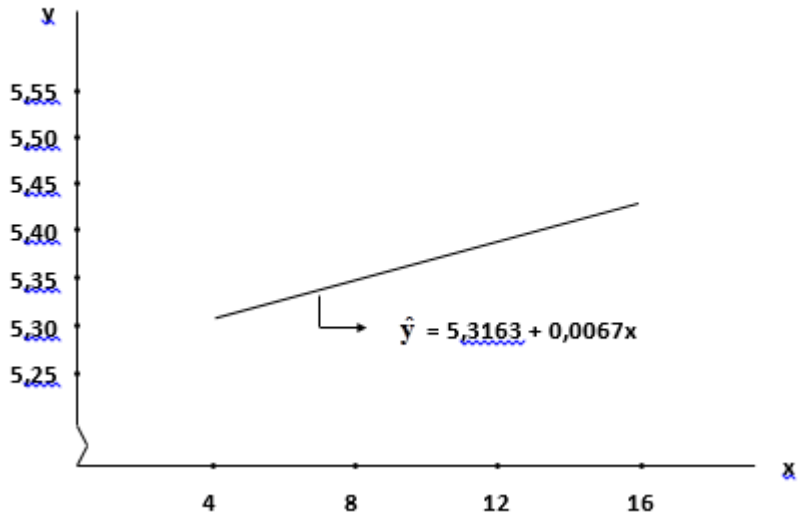

Figure 4. Graph of the Relationship Between Length of Meat Storage at Low Temperature (X) with the Ph Value of Meat (Y)

The increase in the pH value of meat in Figure 4 is due to the increase in the average moisture content of meat during storage from 4 to 16 days (Table 1). This may occur because free water molecules between protein molecules are easily separated so that during meat storage from 4 to 16 days the percentage of free water increases, which means the pH value of the meat also increases. Praktiknya et al. (2023) stated that meat that is stored for too long will lose bound water into free water. The more free water in the meat, the more damaged the meat condition is. This damage is caused by the growth of microorganisms due to the large amount of free water..

Colony Counts of Beef Lactic Acid Bacteria

The observation data for the effect of treatment using L. plantarum culture and glycerol on beef stored at low temperature (4°C) for 4 to 16 days on the mean number of lactic acid bacteria are listed in Table 3.

Table 3. Mean Colony Counts of Lactic Acid Bacteria in Beef After Feeding L. Plantarum and Glycerol and Stored at Low Temperature (4°C) for 4 Days, 8 Days, 12 Days, and 16 Days

***L. plantarum* culture and glycerol (A)**	**Length of Storage Days (B)**				**Average**
	4 (B_1)	**8 (B_2)**	**12 (B_3)**	**16 (B_4)**	
A_1 (10% + 0%)	6,26	6,23	6,32	6,22	**6,26**
A_2 (10% + 5%)	6,29	6,32	6,38	6,20	**6,30**
A_3 (10% + 10%)	6,28	6,36	6,38	6,31	**6,33**
A_4 (10% + 15%)	6,34	6,35	6,40	6,31	**6,35**
Average	**6,29**	**6,31**	**6,37**	**6,26**	**6,31**

Table 3 shows that the addition of glycerol from 5 to 15% in L. plantarum 10% culture caused an increase in the number of lactic acid bacteria colonies. While the length of storage at low temperature caused an increase in the number of lactic acid bacteria colonies until 12 days of storage then decreased at 16 days of storage.

The results of analysis of variance for factor A showed that the use of L. plantarum 10% culture and glycerol in beef had a very significant effect (P < 0.01) on the number of lactic acid bacteria. Similarly, factor B showed that the length of beef storage at low temperature had a very significant effect (P < 0.01) on the number of lactic acid bacteria colonies in beef.

Further analysis with orthogonal polynomial comparisons for factor A showed that the addition of 5 to 15% glycerol in the 10% L. plantarum culture used in beef had a very significant (P < 0.01) linear effect on the number of lactic acid bacteria colonies. The linear effect on the number of lactic acid bacteria colonies mathematically followed the equation = 6,2863 + 0,0032x or more clearly can be seen in the linear response curves listed in Figure 5.

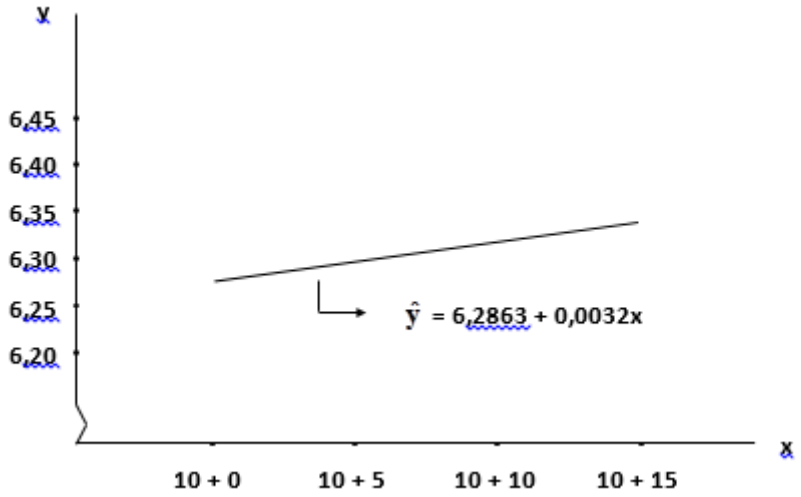

Figure 5. Relationship Graph Between 10% L. Plantarum Culture and Glycerol Levels 0 to 15% (X) with the Number of Colonies of Beef Lactic Acid Bacteria (Y)

The increase in the number of lactic acid bacteria in beef as shown in Figure 5 indicates an increase in the number and activity of lactic acid bacteria to produce lactic acid, as a result of the addition of 5 to 15% glycerol in the 10% L. plantarum culture used in beef. This can occur due to a decrease in pH value or an increase in lactic acid levels in the meat, allowing the development of lactic acid bacteria. As stated by Dwyana (2005); Isnafia (2016); Siswara (2019) that lactic acid bacteria are a group of bacteria that produce large amounts of lactic acid that will reduce the pH value of their growth environment.

Orthogonal polynomial comparisons for the effect of factor B showed that the length of beef storage at low temperature had a very significant (P < 0.01) quadratic effect on the number of lactic acid bacteria colonies. The quadratic effect on the number of lactic acid bacteria colonies mathematically follows the

equation = 6,1529 + 0,0394x – 0,0020x2 or more clearly can be seen in the quadratic response curves listed in Figure 6.

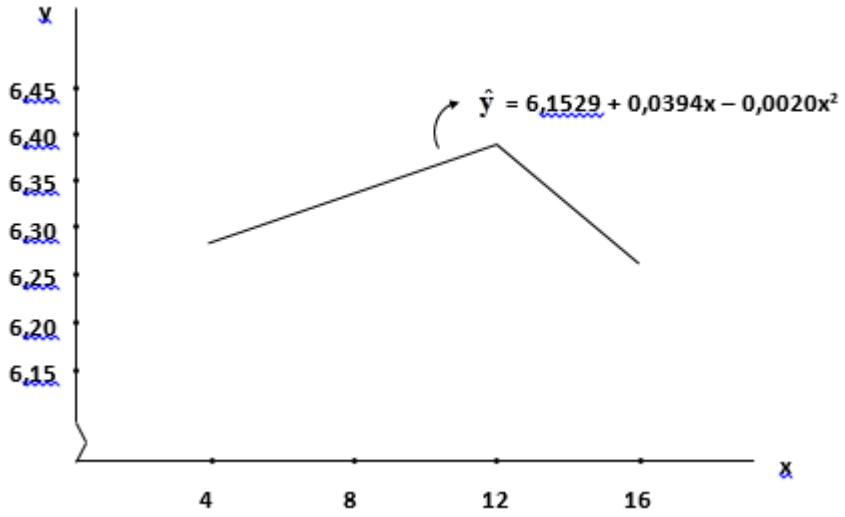

Figure 6. Graph of the Relationship Between Length of Beef Storage at Low Temperature (X) with the Number of Colonies of Lactic Acid Bacteria (Y).

The quadratic response curve listed in Figure 6 shows that lactic acid bacteria in beef stored at low temperatures can still experience increased growth until 12 days of storage and a decrease in lactic acid bacteria during 16 days of storage. This is because lactic acid bacteria are already in the final phase of growth, so the speed of bacterial division decreases and the number of dead bacteria increases. And also because the substances used are decreasing, as well as the hoarding of toxic substances, all of which affect the physiological activity of bacteria (Hariani, 2013; Praktiknya, 2023)

The variety of AB factors is not significantly different, indicating that the effect of adding glycerol at levels of 5 to 15% in L. plantarum 10% culture is the same at 4 days, 8 days, 12 days, and 16 days of storage.

CONCLUSION AND RECOMMENDATION

Based on data analysis and discussion for all variables, it can be concluded that the use of 10% Lactobacillus plantarum culture and 15% glycerol is the maximum level in maintaining the quality of beef stored for 16 days at low temperature (4°C).

FUTHER STUDY

This research still has a delay, so it is necessary to conduct further research related to the topic of Use of Lactobacillus Plantarum Culture and Glycerol in Beef Stored at Low Temperature to improve this research and add insight for readers

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