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## Effect of Incubation Time on Chicken Egg Fermentation Using *Lactobacillus plantarum*

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### **Abstract:**

Eggs are fragile foodstuffs because they are fragile and contaminated with microbes. Utilization of *Lactobacillus* type bacteria commonly used in food products is *Lactobacillus plantarum*. This study used a Completely Randomized Design (CRD) factorial pattern with an incubation time treatment, 90 fresh eggs obtained from the same chicken farm, *Lactobacillus plantarum* FNCC 0027 bacterium and each treatment was repeated 3 times. Parameters measured in this study were lactic acid (%), pH value and total protein (%). The results showed an increase in lactic acid levels and decreased pH value and total protein as the incubation time increased. The fermented chicken eggs have used the optimum *Lactobacillus plantarum* at 37°C for 96 hours of incubation.

**Keywords:** Chicken Egg, *Lactobacillus plantarum*, Fermentation, Incubation Time.

### **1. Introduction**

Eggs are farm products that contain nutrients that are complete and easily digested. Eggs are in great demand because eggs are an inexpensive source of animal protein and are readily available to all societies. Eggs also have balanced amino acids, essential fats, some minerals and vitamins, but the eggs are fragile foodstuffs because they have microbiology and microbial contamination. One way to improve egg storage is through the process of fermentation technology. Fermentation technology is performed to benefit as functional food is good for health, facilitate digestion, absorption and to extend the shelf life of the product. Fermentation technology in foodstuffs using microbes has been widely practiced as *Lactobacillus* type bacteria. Utilization of *Lactobacillus* type bacteria commonly used in food products is *Lactobacillus plantarum*.

Various studies have been conducted on the application of fermentation technology to eggs. The research produced a great prospect of this fermentation technology. The growing ability of *Lactobacillus plantarum* in egg whites and infertile eggs improves egg quality. The ability of *Lactobacillus plantarum* can be seen in lactic acid levels and the resulting pH value as well as the ability of bacteria to degrade the proteins found in eggs, but *Lactobacillus plantarum* requires longer adapted to grow in eggs so it is necessary to study the optimal time of *Lactobacillus plantarum* to grow at Egg medium. Based on the study authors then conducted research on the influence of incubation time to eggs fermented chicken using *Lactobacillus plantarum*.

### **2. Materials and Methods**

This study used a material consisting of 90 chicken eggs derived from the same farm, the amount is calculated based on the total treatment unit amounting to 3 treatments with 3 replications, each treatment unit requires 10 eggs of chicken, lactic acid bacteria culture (BAL) *Lactobacillus plantarum* FNCC 0027 MRS (Man Rogosa Sharpe) broth, aluminum foil, tomato juice, aquades, alcohol, NaOH 0.1 N, PP (phenolphthalein), buffer solution pH 4 and pH 7, K<sub>2</sub>SO<sub>4</sub>, HgO, H<sub>2</sub>SO<sub>4</sub>, NaOH-thiosulfate.

#### **2.1. Culture Propagation**

*Lactobacillus plantarum* FNCC 0027 is stored on De Man Rogosa Sharpe (MRS) agar medium. Propagation of culture by making subculture. Subculture production is done by transferring culture stock to the liquid medium of MRS broth, which has added 20% tomato extract and incubated for 24 hours. Cultures stored on MRS broth medium were inoculated as much as 10% into an egg white containing 20% tomato extract to produce a working culture (Nahariah *et al.*, 2013).

## 2.2. Sample Preparation

The egg samples were cleaned and pasteurized at 60°C for 3,5 minutes (Froninget *al.*, 2002) then separated from the shell then put into the sample bottle. The 100-ml sample was homogenized and subsequently sterilized using ultraviolet by placing it in PCR Hood for 15 minutes. The sterile sample was added to a working culture of 10 ml and subsequently homogenized with a tube shaker, the sample subsequently fermented at 37°C during the study treatment (Nahariah *et al.*, 2015).

## 2.3. Lactic Acid Level (titration method)

The acidity can be analyzed by the titration method by 10 ml suspension plus three drops of phenolphthalein, then titrated with 0,1N NaOH solution. The formula used is:

$$\% \text{ Lactic Acid} = \frac{\text{ml NaOH} \times \text{N NaOH} \times 0,09}{\text{Weight of sample}} \times 100 \%$$

## 2.4. Measurement of pH

The pH meter is ignited and neutralized for 15 - 30 minutes and standardized with buffer solution pH 4 and pH 7. The pH meter electrode is then rinsed with aquades and then dried with tissue paper. The sample can be measured after the pH meter is calibrated. The pH meter is dyed in the sample and then left until the pH meter is stable. The value is shown on the pH meter, monitor screen. After the measurement, pH meter, then rinsed with arcades and dried with a tissue.

## 2.5. Total Protein Content

2 ml sample was put into a Kjeldahl flask together with 20 g of K<sub>2</sub>SO<sub>4</sub>, 1 g HgO and 25 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. Pumpkin then boils until a clear solution is formed and continued for 30 minutes. Pumpkin was allowed to cool and gradually added 200 ml of arcades until the temperature reached 25°C. Adding the NaOH-thiosulfate solution carefully to prevent sudden mixing. Connect the flask with the distillation apparatus and heated until all ammonia is distilled. The remaining standard acid solution is titrated with a standard NaOH solution.

## 2.6. Data Analysis

The data obtained in this study were processed by Variety Analysis based on Completely Randomized Design (CRD) with three treatments of incubation time is 0, 48, and 96 hours. Each treatment is repeated 3 times.

## 3. Result

The results of the study on lactic acid levels, pH value and total protein of fermented chicken eggs using *Lactobacillus plantarum* at different incubation times are presented in Table 1.

Incubation Time (Jam)	Lactic Acid Level (%)	pH value	Total Protein (%)
0	0,402±0,05 <sup>a</sup>	7,00±0,19 <sup>a</sup>	10,86±0,11 <sup>a</sup>
48	1,703±0,08 <sup>b</sup>	5,89±0,17 <sup>b</sup>	10,64±0,04 <sup>b</sup>
96	2,073±0,12 <sup>c</sup>	4,45±0,10 <sup>c</sup>	10,49±0,04 <sup>c</sup>
Average	1,393±0,08	5,78±0,15	10,66±0,06

Table 1: Lactic Acid Level, pH and Total Protein of Chicken Egg with Different Incubation Time  
Description: Different superscript abuses in the same column show significant differences (P 0.01).

### 3.1. Lactic Acid Level

Analysis of variance showed that incubation time had a significant effect (P 0,01) on lactic acid level in egg of racial chicken. Lactic acid levels increase with increasing incubation time. The optimum lactic acid content of the chicken eggs at 37°C incubation temperature for 96 hours' incubation time.

### 3.2. pH value

Analysis of variance showed that incubation time had a significant effect (P 0,01) to pH value on chicken egg. The pH value decreases as the incubation time increases. The optimum pH value of the chicken eggs at 37°C incubation temperature for 96 hours' incubation time.

### 3.3. Total Protein

The analysis of variance showed that incubation time had a highly significant effect (P 0.01) on total protein in chicken eggs. Total protein decreases with increasing incubation time. The optimum pH value of the chicken eggs at 37°C incubation temperature for 96 hours' incubation time.

## 4. Discussion

### 4.1. Lactic Acid Level

Treatment of different incubation times in eggs as growth medium of *Lactobacillus plantarum* showed elevated lactic acid levels in line with increasing incubation time. Increased incubation time also increases the number of *Lactobacillus plantarum* to produce higher lactic acid as a result of fermentation. Primurdia and Kusnadi (2014), The growth of lactic acid bacteria is increasing able to remodel components - components of sugar in the medium to the maximum, so the lactic acid bacteria metabolite calculated as total lactic acid increased, so the degree Acidity on the characteristics of the final product may also decrease.

The production of lactic acid during the incubation period has increased due to an increase in the number of bacteria at an increased incubation time which can break down carbohydrate and protein compounds in foodstuffs. Nahariah *et al.* (2013), the difference in the percentage of the total acid of each foodstuff is determined by the ability of the microbes to break down the constituent components of the food. The utilization of *Lactobacillus plantarum* bacteria can increase the acidity by 1.5 to 2.0% (Afriani *et al.*, 2011).

#### 4.2. pH value

The treatment of different incubation times in eggs as growth medium of *Lactobacillus plantarum* showed a decrease in pH value in line with increasing incubation time. This occurs because the increase in incubation time also increases the amount and metabolism of bacteria that produce lactic acid so that the pH value decreases. Nisaet *al.* (2008), lactic acid as the main product of easily dissociated fermentation produces  $H^+$  and  $CH_3CHOHCOO^-$ . The presence of  $H^+$  ions further influences the pH value, the more lactic acid produced the  $H^+$  ion concentration increases and is measured at the pH value. The accumulation of lactic acid produced by lactic acid bacteria can decrease the pH of the fermentation medium. The calculated pH value is the liberated  $H^+$  concentration during the fermentation process. Nahariah *et al.* (2013), the decrease in pH is due to the presence of fermentation activities that convert carbohydrates or sugars in foodstuffs to acid and water and other end products.

#### 4.3. Total Protein

Total protein in chicken eggs decreases as the incubation time increases. This is due to the high number of bacteria that break down the protein during fermentation so that the total protein decreases. Chalid and Elmer (2008), the decrease in total protein occurs suspected because during fermentation, a reshuffle of proteins into amino acids and peptides (simpler compounds) by microbes. The content of amino acids and protein peptides in the fermentation medium can affect the total protein decline during fermentation. Nadra (2007), the decrease in total protein is increasing with the increasing number of total lactic acid bacteria, resulting in more and more amino acids and peptides is formed. Protein in the form of Arginine, can be utilized as an additional energy source in *Lactobacillus plantarum*.

### 5. Conclusion

The use of chicken eggs as a medium for growth of *Lactobacillus plantarum* bacteria at incubation temperature of 37°C for 96 hours' incubation time can increase the lactic acid level and decrease the pH value and total acid.

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