

Physical Quality of Cow's Milk by Exposure to Magnetic Fields Extremely Low Frequency (ELF) 300 μ T and 500 μ T by inhibiting *Salmonella* and *Escherichia Coli* Growth

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ABSTRACT

Extremely Low Frequency (ELF) magnetic field, high intensity (more than 100 μ T), can suppress the growth of pathogenic bacteria, but the impact of low intensity ($\leq 500 \mu$ T) needs to be proven. This study aimed to analyze the physical resistance of cow's milk exposed to a magnetic field of 300 μ T and 500 μ T ELF by inhibiting the proliferation of *Salmonella* and *Escherichia Coli* bacteria. Completely Randomized Design (CRD) research design. Samples were 210 bottles of cow's milk (@ 50 ml) divided into seven groups. Exposure to the ELF magnetic field intensity of 300 μ T and 500 μ T with exposure variations of 60, 90, and 120 minutes. Result: Exposure to an ELF magnetic field with an intensity of 500 μ T for 60 minutes significantly ($p < 0.05$) reduced the *Salmonella* sp. population but not *Escherichia coli*. Even though the physical condition showed the color and smell of fresh milk, lumps did not appear until the 10th hour. Conclusion: exposure to the ELF magnetic field intensity of 300 μ T and 500 μ T has not been able to maintain the quality of cow's milk.

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I. Introduction

Milk is a liquid obtained from the udders of healthy and clean cows by proper milking, where the natural content is not reduced or added to an ingredient and has not received any treatment except cooling [1]. The high nutritional content makes milk a good medium for the growth of microorganisms so that in suitable environmental conditions, bacteria will proliferate in milk [2]. Based on research by Subagyo *et al.* [3] proved that fresh cow's milk in the sub-districts of Sumbang and Baturaden has a shelf life of about 6.5 hours. According to Zakaria *et al.* [4], improper handling of milk can reduce the shelf life of fresh cow's milk. The rapid development of bacteria in milk causes milk spoilage and is inappropriate for consumption [5]. The increase in the number of bacteria in milk makes the activity of lactic acid spoilage bacteria increase the production of lactic acid [6],

so it will increase the acidity in the milk, and the pH value will also decrease [5], [7]–[9]. SNI 3141.1: 2011 shows that good milk has a degree of acidity (pH) of 6.3–6.8 [1]. Cow milk is in a fresh state if the milk does not change color, smell, or viscosity [10], [11]. A decrease in pH value causes coagulation of milk protein (casein) [12]. Increased casein coagulation will increase the viscosity (thickness), which will affect the density of milk.

Many milk preservation techniques have been applied to increase the shelf life of milk, including pasteurization and cooling. Pasteurization is a milk preservation technique by heating [13]. The pasteurization process can reduce nutrients and nutrients due to the protein denaturation process [14]. Preservation techniques by cooling cannot kill bacteria but only inhibit the growth of bacteria [15], [16]. Thus, other alternatives are needed in handling and processing fresh cow's milk.

Exposure to high-intensity ELF magnetic fields ($> 500 \mu\text{T}$) suppresses the growth of pathogenic bacteria. Sudarti [17] reported that the intensity of the ELF magnetic field of $646.7 \mu\text{T}$ for 30 minutes suppresses the *Salmonella typhimurium* population in gado-gado seasoning by up to 56% and 17% in the vegetables. The ELF magnetic field at an intensity of $730 \mu\text{T}$ for 2×30 minutes can reduce bacterial growth in milkfish by up to 73% and at an intensity of $880 \mu\text{T}$ by up to 62% [18]. At the same time, Sari *et al.* [19] reported that the intensity of the ELF magnetic field of $700 \mu\text{T}$ with an exposure time of 30 minutes was able to maintain the pH of chicken meat. However, it is still necessary to prove the effect of exposure to low-intensity ELF magnetic fields ($\leq 500 \mu\text{T}$) on food security. Therefore, this study aims to analyze the physical resistance of cow's milk exposed to Extremely Low Frequency (ELF) $300 \mu\text{T}$ and $500 \mu\text{T}$ magnetic fields.

II. Theory

Extremely Low Frequency (ELF) magnetic field radiation can penetrate almost any material, including biological material such as bacteria. The ELF magnetic field force will act on the ions in the cell membrane so that the ions will be polarized and impact increasing calcium ions in the cell nucleus. If the increase in calcium in the cell nucleus is high enough, it will weaken cell function or cell death. This condition will impact the resistance of bacteria to become weak or even die [20]. Exposure to magnetic fields will damage the proteins in cells so that cell nutrition or organic nutrients in the form of proteins that play a role in intracellular metabolic mechanisms will be damaged [21], [22]. Pathogenic bacteria, especially *Salmonella sp* and *Escherichia Coli*, affect the process of food spoilage, including cow's milk. Exposure to low-intensity ELF magnetic fields ($< 500 \mu\text{T}$) is expected to inhibit the proliferation of these pathogenic bacteria so that milk quality will last longer. The cell membrane is the initial interaction medium for the ELF magnetic field with living things, including bacteria, but the impact will differ for different bacteria types. This is because each strain of bacteria responds differently depending on the frequency, intensity, and duration of exposure to the ELF magnetic field [23].

A study by Sari *et al.* [19] proved that the ELF magnetic field with an intensity of $700 \mu\text{T}$ and a long exposure of 30 minutes can maintain the pH of chicken meat until the 10th hour. Research by Kristinawati and Sudarti [24] reported that the intensity of exposure to the ELF magnetic field of $100 \mu\text{T}$ for 5 minutes could reduce the water content in making cream cheese. This indicates that the ELF magnetic field might be able to increase the shelf life of food ingredients, so researchers are inspired to increase the shelf life of fresh cow's milk using exposure to the ELF magnetic field. The ELF magnetic field is a component of ELF electromagnetic waves with frequencies between 0-300 Hz [25]. The ELF magnetic field includes non-ionizing and non-thermal radiation, so it is not potentially toxic or damaging and will not lose or

damage its nutrients [20]. Exposure to the ELF magnetic field also does not cause damage to chemical bonds and DNA [26].

III. Method

The type of research used is experimental research with a factorial design. Research data were analyzed using quantitative statistical analysis with a different statistical test using *Microsoft Office Excel 2007* application and *IBM SPSS Statistics 22*. The sample was fresh cow's milk with a total of 210 sample bottles for measuring pH values and density and observing physical conditions and 30 bottles. Samples for bacterial observation with each sample bottle with a volume of 50 ml milk. The input voltage of the ELF magnetic field used is 220 V with a frequency of 50 Hz and a current of 5 A. The research procedure consists of 5 stages: preparation, treatment, storage, measurement, and observation. Preparatory steps: 1) Prepare research tools and materials. 2) Determine and divide the samples, with each sample as much as 50 ml, into a plastic bottle.

At the treatment *stage*, the experimental group carried out exposure to the ELF magnetic field with an intensity of $300 \mu\text{T}$ and $500 \mu\text{T}$ for 60, 90, and 120 minutes. Furthermore, at the storage stage, all samples were stored at room temperature for 5, 10, and 15 hours after exposure. The steps for measuring pH values and density and observing physical conditions were carried out at 5, 10, and 15 hours after exposure. The pH value was measured by dipping the pH meter electrode into each sample, then left until a stable value was obtained on the pH meter screen. Density measurements were carried out by dividing the volume by the mass of each fresh cow's milk sample using a measuring cup, pycnometer, and digital balance.

Bacterial observations were carried out using EMBA media for *Escherichia coli* bacteria and SSA for *Salmonella sp* bacteria at 29, 31, and 33 hours after exposure by planting bacteria 3 and 6 hours after exposure. Each sample was planted three times so that the total number of Petri cups used was 90 plates for SSA media and 30 for EMBA media. The agar media was prepared by dissolving and boiling 81 grams of SSA media and 48.6 grams of EMBA media in 1350 ml of distilled water. Next, the boiling SSA and EMBA media were poured into Petri dishes and allowed to cool. The bacteria planting stage is carried out by 1) Sterilizing the researcher's place and hands with an alcohol solution, 2) A total of 1 ml of milk sample was dissolved in 9 ml of distilled water and shaken using a vortex (10^{-1} dilution and repeat until 10^{-6} dilution), 3) 1 ml of the sample suspension solution at a dilution of 10^6 was poured and leveled on SSA and EMBA media, 4) Cover and heat the Petri cup with a spirit burner and seal it again with plastic wrap, 5) Incubation for 24 hours, 6) Observe colonies of *Escherichia coli* and *Salmonella sp* on SSA and EMBA media.

IV. Results and Discussion

Changes in the pH of Cow's Milk

The pH values of cow's milk in the control and experimental groups at 0, 5, and 15 hours are shown in Figure 1.

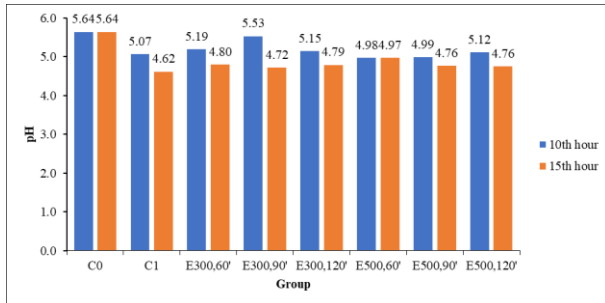


Figure 1. The pH value of the Research groups at the 10th and 15th hours

Samples of cow's milk with a pH of 5.64 decreased after exposure to the ELF magnetic field at an intensity of 300 μ T and 500 μ T. Data on measuring the pH of cow's milk at 10 and 15 hours showed that the pH of all groups decreased. However, only the group exposed to the ELF magnetic field at 300 μ T intensity for 90 minutes had a significantly ($P < 0.05$) higher pH value than the control or other groups.

Data analysis begins with the normality test, and the normality test output shows the Asymp. Sig. (2-tailed) of 0.000 or < 0.05 so that the data is not normally distributed. Therefore, quantitative statistical analysis was carried out with the Independent Sample t-test – Mann-Whitney test. Based on the output of the Independent Statistical test Sample t-test – Mann-Whitney test, it can be seen that at 5 hours after exposure to the Asymp. Sig. (2-tailed) between experimental control groups (E300-60', E500-60', E500-90', and E500-120') < 0.05 so that H_0 is rejected and H_a is accepted. While the value of Asymp. Sig. (2-tailed) between the control and experimental groups (E300-90' and E300-120') > 0.05 so that H_0 is accepted and H_a is rejected. At 10 hours after exposure, the Asymp. Sig. (2-tailed) between control and experimental groups (E300-60', E300-90', E500-120', E500-60', and E500-90') < 0.05 , so H_0 is rejected, and H_a is accepted. While the value of Asymp. Sig. (2-tailed) between the control and experimental groups (E500-120') > 0.05 so that H_0 is accepted and H_a is rejected. At the 15th hour after the exposure of the Asymp. Sig. (2-tailed) between the control group and the entire experimental group < 0.05 so that H_0 is rejected and H_a is accepted.

Based on the output of the Kruskal Wallis test, it showed significantly ($p < 0.05$) the pH value of the group exposed to the ELF magnetic field was lower than the control group at 5, 10, and 15 hours. The decrease in pH value was thought to be due to bacterial activity in milk which converts lactose sugar into lactic acid so that the acidity of the milk increases [9]. The increase in acidity causes the concentration of hydrogen ions (H^+) to be

higher, decreasing the pH value [27]. Microbial contamination in milk occurs from milking and collecting milk to packaging [24]. Exposure to the ELF magnetic field causes the growth of bacteria to be inhibited by transferring energy from the magnetic field to the ions in acid-forming bacteria so that bacterial cells will die [20]. According to Barbosa and Canovas [21], the provision of a magnetic field will cause the ionization of some salts, such as Mg^+ and Ca^{2+} , which are bound to the cell wall. If the cell is deficient in calcium ions, it will inhibit its function in the cell.

The inhibition of bacterial activity by exposure to the ELF magnetic field causes the production of lactic acid by bacteria to be inhibited so that the decrease in pH value can be maintained. This has been proven by the research results showing that the control group sample from 0 to 15 hours after exposure experienced a drastic decrease in the average pH value compared to the experimental group. Thus, exposure to the ELF magnetic field affects changes in the pH value of fresh cow's milk. The inhibition of bacteria by exposure to the ELF magnetic field has also been demonstrated by research [28] which proves that exposure to the 0.3 Hz QAMW ELF magnetic field for 90 minutes can inhibit the growth of *Escherichia coli* bacteria up to 42.3%.

Changes in the Density of Cow's Milk

The density of cow's milk in the control and experimental groups at the 10th and 15th hours is shown in Figure 2.

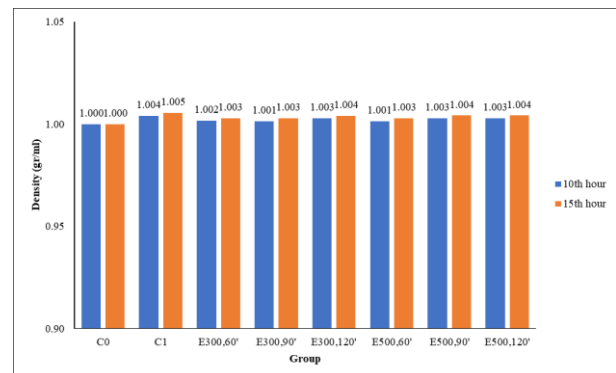


Figure 2. The density of the Experimental at the 10th and 15th hours

Based on Figure 2, the average density of fresh cow's milk in the control and experimental groups from 0 to 15 hours after the above exposure shows that the longer the storage time, the higher the density. This follows research by Roza and Aritonang [29], which proves that the longer the milk storage will increase the density caused by fat compaction. According to [30] Density of milk will increase according to storage time. Figure 2 shows that at 0 to 15 hours after exposure, the control group sample experienced a very drastic increase in density. The experimental group sample (E300-120') experienced a drastic increase. Samples of the E300-60' and E500-60'

groups experienced a significant increase in density, with the E300-60' group having a higher density increase than the E500-60' group at 5 hours after exposure. Therefore, it can be stated that exposure to the ELF magnetic field at an intensity of 500 μT with an exposure time of 60 minutes is optimal for maintaining the density of fresh cow's milk. The results of the normal distribution test showed that the data on the density of fresh cow's milk was not normally distributed ($p < 0.005$), so a non-parametric analysis was performed using the Mann-Whitney test. The Mann-Whitney statistical test results showed no significant difference ($p > 0.05$) between the control group and all sample groups exposed to ELF magnetic fields with either 300 μT or 500 μT intensity. There was no difference in density between the control and treatment groups at 5, 10, and 15 hours. The possibility of a change in density that occurred was so small that the difference in density in each treatment group was not visible.

An increase in the number of bacterial colonies in the milk caused an increase in fresh cow's milk density. The higher number of bacterial colonies will cause acidity and fat compaction to increase. The increase in acidity and compaction of fat causes the clumping of milk, so the density of milk will also increase [29]. The occurrence of coagulation of milk protein (casein) is due to a decrease in acidity in the milk. The increase in casein coagulation causes milk coagulation to increase so that the viscosity of milk also increases [12]. Increasing viscosity will affect the value of the density. That is, the value of the viscosity is directly proportional to density [31].

Cow's Milk Physical Resistance

The Smells of Cow's Milk

The results of observing the physical condition smell of cow's milk in the control and experimental groups at the 10th and 15th hours after exposure are presented in Figure 3. Figure 3 shows that at 5, 10, and 15 hours after exposure, it shows that the smell of the control group sample experienced a bigger change than the experimental group and indicated that the experimental group with an intensity of 500 μT with an exposure time of 60 minutes had the potential to inhibit smell changes fresh cow's milk. The change in the smell of cow's milk from very flavorful to cow's milk to a pungent rotten smell is caused by bacteria growing due to acid. According to Aritonang [32], bacteria that cause a bad smell in milk are included in spoilage bacteria, including *Bacillus fluorescens* and *Bacillus subtilis*. These bacteria will break down the milk casein *Proteus* to form compounds that can cause a rotten odor [33]. The change in the aroma score of cow's milk in the experimental group was smaller than the change in the aroma score in the experimental group because the experimental group was able to inhibit and suppress bacterial growth so that the decay process was inhibited and able to maintain the aroma of milk.

The Lumps of Cow's Milk

The next physical condition indicator observed was lumps. Data from the observation of lumps of fresh cow's

milk samples at the 10th and 15th hours after exposure can be seen in Figure 4.

The score of cow's milk lumps at 10 hours significantly decreased in the group exposed to the ELF magnetic field intensity of 300 μT and 500 μT but was not different from the control. It can be seen that the score of cow's milk lumps in the sample group exposed to an intensity ELF magnetic field of 500 μT was better at 15 hours than the control.

This shows that the intensity of exposure to a magnetic field of 500 μT is more optimal for maintaining clumps than the experimental group with an intensity of 300 μT . Thus, optimal exposure to the ELF magnetic field can suppress bacterial growth. Clumps in milk are caused by the proliferation of spoilage bacteria that attack casein, causing a foul odor [32]. The longer storage of milk causes the development of bacteria faster, so lumps will also be thicker.

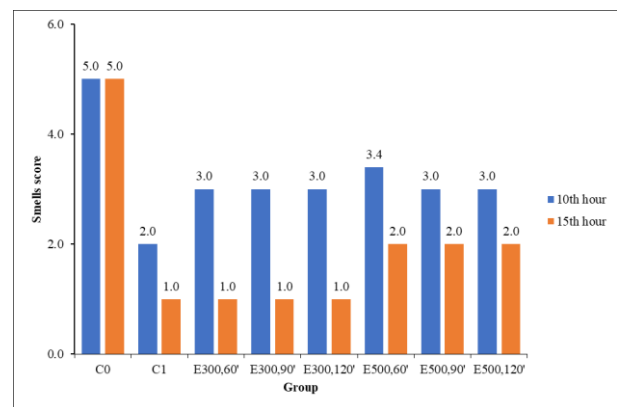


Figure 3. The average Smells score of cow's milk at the 10th and 15th hours

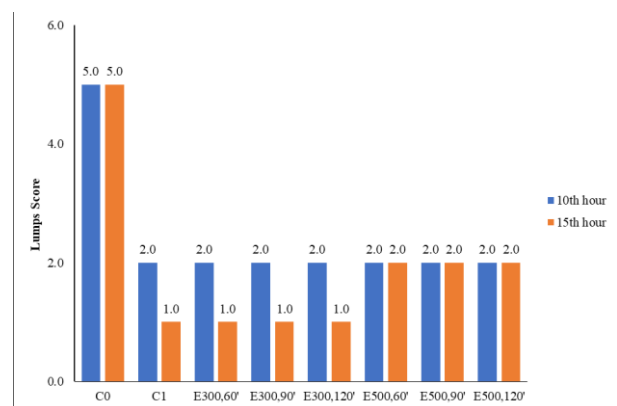


Figure 4. The average Lumps Score of cow's milk at the 10th and 15th hours

The Colors of Cow's Milk

Observation data for the colors of fresh cow's milk samples at the 10th and 15th hours after exposure can be seen in Figure 5. The observational data and the color diagram of cow's milk show that at the 10th and 15th hours after exposure, there is no change in color from the physical condition of the initial color of the fresh cow's

milk sample, namely, the color of cow's milk is yellowish white. Normally, cow's milk is white and slightly yellowish. The white color in milk is obtained from colloidal particles of milk protein compounds (casein), calcium phosphate, and fat globules. The yellow color in milk is obtained from the pigment carotene and riboflavin dissolved in milk fat [34].

The population of *Salmonella* sp and *Escherichia Coli* bacteria

The growth of pathogenic bacteria, namely *Salmonella* sp and *Escherichia coli* bacteria, was measured 3 hours after the sample was exposed to the ELF magnetic field. Ways to detect *Salmonella* sp bacteria with SSA media (*Salmonella* shigella agar) and to detect E-coli with EMBA (Eosin Methylene Blue Agar) media in cow's milk samples. Furthermore, bacterial growth was observed at 31 and 33 hours by calculating the bacterial population. Based on the normal distribution test on *salmonella* sp and *Escherichia coli* bacteria population data in milk samples in 7 study groups, it proves that the data is not normally distributed. Therefore, statistical analysis using non-parametric with the Mann-Whitney test followed by the Kruskal Wallis test.

The proliferation of Salmonella bacteria

The following shows a graphic display of the population of *Salmonella* sp bacteria in the 7 study groups (see Figure 6). Figure 6 illustrates the average number of *Salmonella* sp colonies in 7 study groups. It can be seen that exposure to an ELF magnetic field intensity of 300 μ T with an exposure time of 60 minutes and 90 minutes and exposure to an ELF magnetic field intensity of 500 μ T for 60 minutes can suppress the proliferation of *salmonella* bacteria.

The results of the Kruskal Wallis analysis proved that the population of *Salmonella* sp bacteria in the sample group of cow's milk was exposed to an ELF magnetic field intensity of 300 μ T for 60 minutes and 90 minutes. The sample group of cow's milk was exposed to an ELF magnetic field intensity of 500 μ T for 60 minutes. Significantly ($p < 0.05$) lower than the control and other groups.

Salmonella sp are pathogenic bacteria that are harmful to human health. In general, *Salmonella* grows actively at a pH between 3.6-9.5 and is optimal at a pH close to normal [33]. It has been reported that exposure to the ELF magnetic field (500 μ T, 50 Hz) for 120 minutes has been shown to suppress the proliferation of *salmonella* sp on vannamei shrimp [35].

The proliferation of Escherichia coli bacteria

Data on the results of measuring the population of *Escherichia Coli* bacteria in the 7 study groups are described in Figure 7. Figure 7 illustrates the average number of *Escherichia coli* colonies in the 7 study groups. Based on the results of Kruskal Wallis statistical analysis, it is proven that the population of *Escherichia Coli* colonies in the sample group of cow's milk exposed to an ELF magnetic field intensity of 300 μ T for 60 minutes and the sample group of

cow's milk exposed to an ELF magnetic field intensity of 500 μ T for 60 minutes and 90 minutes significantly ($p < 0.05$) lower than the control and other groups.

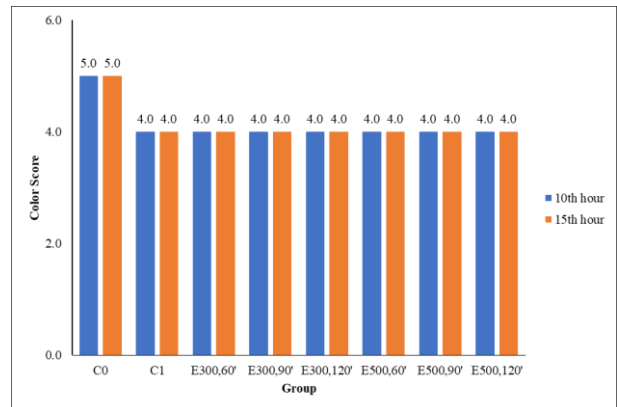


Figure 5. The average color score of cow's milk at the 10th and 15th hours

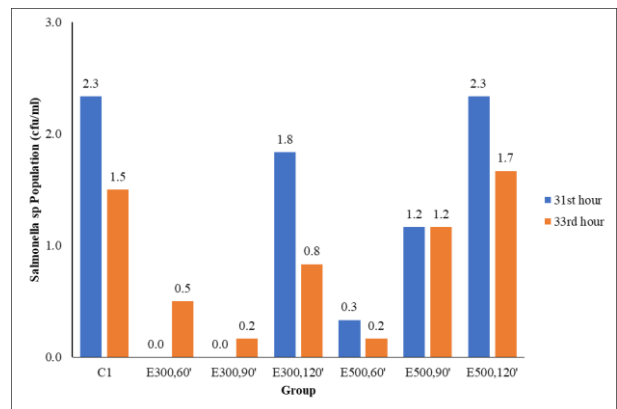


Figure 6. The population of *salmonella* sp bacteria in cow's milk during the 31st and 33rd hours

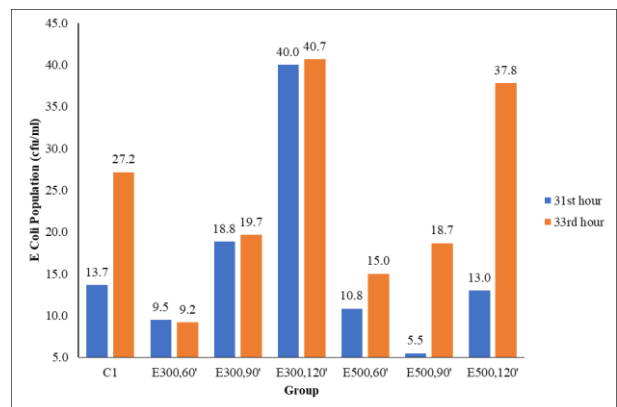


Figure 7. The population of *Escherichia coli* bacteria in cow's milk the 31st and 33rd hours

Several research results have also reported that exposure to the ELF magnetic field with an intensity of 730 μ T with exposure to 2x30 minutes is proven to suppress the proliferation of pathogenic bacteria in milkfish [18]. Exposure to an ELF magnetic field with an

intensity of 1000 μT for 15 minutes increased the resistance of tuna until the 15th hour [36].

This study's results indicate that ELF magnetic field radiation has the potential for the development of bacterial sterilization technology. The ELF magnetic field is non-ionizing radiation with very low energy, so it is relatively safe for food security technology.

V. Conclusion

Extremely Low Frequency (ELF) magnetic field at an intensity of 500 μT could potentially increase the shelf life of cow's milk up to 10 and 15 hours of storage, with a higher cow's milk aroma score than the control. However, only exposure for 60 minutes significantly ($p < 0.05$) was able to suppress the proliferation of *salmonella sp* bacteria and was unable to suppress the proliferation of *Escherichia coli* bacteria.

Based on the results of the research and discussion, the suggestions given are that further research needs to be carried out by further researchers related to exposure to the ELF magnetic field in fresh milk to determine the level of damage to milk with variations in intensity and duration of exposure as well as variations in the independent variables used. In addition, to obtain more accurate research results, researchers should pay more attention to sterilizing research tools, especially sample bottles.

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