

Comparison of Total Number of Bacteria in Raw Milk Friesian Holstein Cow Based on Milking Techniques Using Total Plate Count (TPC) Test

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ABSTRACT

The aim of this research was to know the comparison of total number of bacteria in raw milk based on milking techniques. This research used purposive sampling and used 30 samples, 15 cows from Lucky Farm and 15 cows from three traditional farmers in Medowo Village, Kandangan District, Kediri Regency. All samples were tested using pour plate method Total Plate Count (TPC) test in Veterinary Public Health Laboratory of Veterinary Medicine Faculty, Universitas Airlangga in Surabaya. The existing data will be processed with Kolmogorov normality test and Independent T-test. The result of this research served in tables. The research was conducted in January to February 2022. The result showed that there are some samples have a total number of bacteria that meet predetermined standards, but some other samples do not meet the standards of SNI according to SNI 7388:2009 which is 1×10^6 CFU/ml. The average number of the machine-milked sample was 3.5×10^5 CFU/ml, while in the conventionally milked sample was 2.9×10^6 CFU/ml. The results of the analysis using the Independent T-test showed that there was significant difference in the number of bacteria in milk using machine and conventional milking ($p < 0.05$).

Keywords: raw milk; total plate count; bacteria; FH cow; food production

INTRODUCTION

Milk is referred to as a near-perfect food because of its complete nutritional values. More than 100,000 types of food molecules are found in milk. Milk contains water, fat, carbohydrates, enzymes, proteins, vitamins A, B, C, and D. Milk is needed by humans to build and maintain the body's cells, maintain the body's metabolism, as a source of prebiotics, a source of calcium and to repair damaged tissues¹. Milk contains a low number of bacteria when removed from a healthy udder. However, the number of bacteria can increase when stored for a long time at room temperature (25°C). Milk is an excellent growth medium for bacterial growth. The possibilities that can cause milk to be contaminated with bacteria are water, dirt and contaminating soil during the milking process².

The sources of bacteria that contaminate cow's milk generally come from nipple ducts, infected udder tissue, hand hygiene, contaminated water and feces. Excessive bacterial contamination in milk can cause several diseases including dysentery, typhoids, tuberculosis, and salmonellosis. Therefore, it is important to maintain the safety and quality of dairy milk³. The condition of the cleanliness of the cow showed greatly affects the content of microbial contamination in cow's milk. Microbiological quality of fresh milk in Krucil District, Probolinggo Regency Indonesia has an average TPC of 7.4×10^5 cfu/ml, the number of *Enterobacteriaceae* contamination 7.5×10^2 cfu/ml and *Staphylococcus aureus* contamination 7.9×10^1 cfu/ml⁴.

Two different milking techniques between milking machines and traditional hand milking are certainly very influential on the minimum number of bacteria required in milk. The amount of these bacteria greatly affects the quality of milk. The level of hygiene between milking using a machine and traditionally hands milking also greatly affects the number of bacteria in milk. The microbiological quality of raw milk is one of the quality standards that need to be considered⁵.

While still in the udder milk, the milk is still sterile, contamination can occur after coming out of the udder due to several sources of pollutants⁶. Milk contains microbes less than 5×10^3 CFU/ml, and if the milk is placed at room temperature, the number of bacteria in milk will increase, the longer duration milk located in room temperature the higher the number of bacteria present in milk⁷. Based on the National Standardization Agency (BSN) on National Standard (SNI) no. 7388:2009, there is a maximum limit on the total number of microbial contamination allowed in Raw milk which is 1×10^6 CFU / ml. The average cow business in Indonesia is dominated by small-scale farmers who still use conventional milking method by hand. There are also medium-scale and large-scale farmers who already use machines to milk. The two milking methods have differences in the level of cleanliness and the amount of milk that can be milked⁵. The purpose of the study is to compare the total number of bacteria in raw cow's milk between milking using milking machines and traditional hand milking.

METHODS

This research design is explorative laboratory, and the data obtained are processed quantitatively. Cow's raw milk obtained from Lucky Farm and traditional farms Medowo Village, Kandangan Subdistrict of Kediri Regency Indonesia. The study uses the Total Plate Count (TPC) test. The number of samples taken in this study are 30 FH cows, 15 cows from Lucky Farm and 15 cows, each sample is taken as much as 30-50 ml. The samples are taken using a purposive sampling method. Cows selected according to the predetermined criteria are still productive with production of 10-15 liters a day. Sampling is carried out in the afternoon at 14.00-15.00 according to the milking time of the cow.

The materials used in this research are cow's milk samples, aquadest, NaCl 0,9% to make sample dilution, Nutrient Agar (NA). The tools used in the study are measuring cups, beaker glasses, micropipettes, Petri dishes, autoclaves, aluminum foil, incubators, labels, Bunsen, cotton, refrigeration, test tubes, Erlenmeyer tubes and stationery.

Samples are taken from four teats of a cow then put in a sample pot. To minimize bacterial contamination, milk is taken aseptically and then put in a cool box so that the temperature remains at 4°C when taken to the research laboratory⁸. A total of 33.6 grams of Nutrient Agar powder is dissolved in 1.5 liters of aquadest, after heating while stirring until dissolved. Sterilize with autoclave at 121°C for 15 minutes, then cooled the medium at temperature between 45 – 50°C until later used for the pour plate method.

Milk samples were examined for bacterial contamination by Total Plate Count (TPC). Each milliliter of milk is diluted to 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵. The diluter used to dilute the sample is NaCl 0.9%. The standard used to calculate TPC test results is the Standard Plate Count (SPC), which explain show to calculate colonies on a petri dish and select the data used to calculate a sample. Petri dishes that can be used to count colonies in a sample are those containing colonies between 30-300. Colonies are calculated by manual means, if there are colonies merged into one large colonies can be counted as one colony. Colonies that are seen as thick line counted as one colony. Next, the result is written into one number in front of the comma and one behind the comma multiplied by the dilution calculated⁹. The data used as standard plate count (SPC) must follow the rule that the reported result consists of only two numbers, in front of the comma and behind the comma if the next number is equal to or greater than five then it can be rounded up by one number¹⁰.

Statistical analysis

Existing data will be processed with Kolmogorov and Independent T-Test. Statistical analysis using software on the computer is Statistical Program and Service Solution (SPSS) for Windows version 26.

RESULTS AND DISCUSSION

The result of TPC is shown at table 1 and figure 1. There are some samples have a total number of bacteria that meet predetermined standards, but some other samples do not meet the standards of SNI according to SNI 7388:2009. After data is transformed, mean and standard error mean of milk bacteria count based on milking technique are shown at Table 2.

TABLE 1: Result of TPC test

Milking Machine Method	SPC (cfu/ml)	Conventional Milking Method	SPC (cfu/ml)
L1	7.1 x 10 ⁴	D1	1.9 x 10 ⁶
L2	1.2 x 10 ⁵	D2	2.6 x 10 ⁵
L3	2.7 x 10 ⁶	H1	2.2 x 10 ⁷
L4	3.3 x 10 ⁵	H2	2.4 x 10 ⁶
L5	1.1 x 10 ⁵	H3	1.6 x 10 ⁶
L6	4.9 x 10 ⁵	H4	2.0 x 10 ⁵
L7	6.6 x 10 ⁴	H5	2.9 x 10 ⁵
L8	1.1 x 10 ⁵	M1	2.6 x 10 ⁶
L9	2.5 x 10 ⁵	M2	1.7 x 10 ⁶
L10	1.7 x 10 ⁵	M3	2.9 x 10 ⁵
L11	2.6 x 10 ⁵	M4	2.8 x 10 ⁵
L12	3.8 x 10 ⁴	M5	1.6 x 10 ⁶
L13	1.8 x 10 ⁵	M6	2.1 x 10 ⁶
L14	1.6 x 10 ⁵	M7	2.0 x 10 ⁶
L15	2.9 x 10 ⁵	M8	2.7 x 10 ⁶

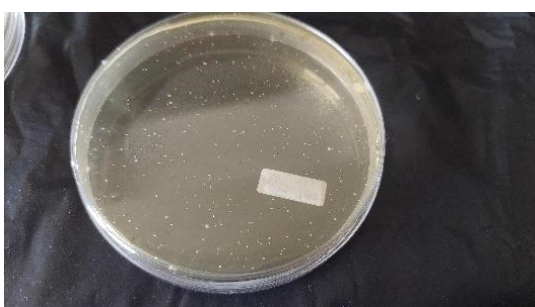


FIGURE 1: Research result dilution 10⁻³ using a milking machine (left), and dilution 10⁻³ using the conventional method (right).

TABLE 2: Mean and standard error mean of milk bacteria count based on milking technique.

Milking Technique	Mean ± SD	p-value
Milking Machine	1.27± 0.43	0.000
Conventional Milking Technique	2.09± 0.55	

While the mean value calculated before the data is transformed by logarithms is 3.5×10^5 CFU/mL for Milking Machine method, while the average number of milk that is milked conventionally is 2.9×10^6 CFU/mL. The results of the analysis using the Independent T-Test showed that there was significant difference in the number of bacteria in milk using machine and conventional milking ($p < 0.05$).

Based on research data, it shows that the total number of bacteria in 30 raw milk samples collected from Friesian Holstein cow's milk from Medowo Village, Kediri were 11 samples exceeded the standards set in SNI 7388:2009. The maximum number of total bacterial counts in milk based on SNI is 1×10^6 CFU/ml (BSN, 2009). In table 1, from 30 samples counted all samples grew bacterial colonies, there were 11 samples consisting of 10 samples in conventional techniques and in a milking machine which sample was 2.72×10^6 CFU/ml, while in the conventionally milked sample was 2.28×10^7 CFU/ml.

The mean value of total bacterial count from conventionally expressed milk samples was higher than that of machine- expressed milk samples. Several factors can cause this to happen, one of which is milk contaminated with bacteria when milking is done by hand, milker did not clean the udder properly and did not do teat dipping. Unhygienic milking equipment, poor milking process, and cage conditions also affect the number of bacteria in milk¹¹.

Bacterial contamination can also occur during handling and sampling. Improper sampling methods can also affect the total number of bacteria in the test. Bacteria need the right environment synthetically as a substitute for natural conditions¹². Handling and sampling must represent at least 99% of the real situation. Containers, tools and materials used for the TPC test must be sterile¹⁰.

Healthy udder should contribute very little to the total bacteria count of bulk milk, a cow with mastitis has the potential to shed large numbers of microorganisms into the milk supply. Infected cows have the potential to shed in excess of 10^7 CFU/ml. Mastitis organisms found to most often influence the total bulk milk count are *Streptococcus sp.*, most notably *S. agalactiae* and *S. uberis*¹³.

Technically, milking can be done with three ways, namely whole hand, stripping and knevelen. The selection of techniques is adjusted for breeders preferences and customized functions. Stripping technique, it is better to use when to spend milk from udder at the end of the milking. Stripping techniques are techniques that have the fastest time efficiency, more milk produced in a period of time that is not as long as the Whole Hand technique, but Stripping techniques can increase the risk of inflammation and mastitis so that it can reduce milk production up to 30%. Most farmers use Whole Hand techniques. The Whole Hand technique is safe, because nipples will not be saggy and avoid inflammation, but it requires a lot of energy¹⁴.

The incorrect milking technique will cause open nipples, and facilitate contaminated bacteria due to contact with the floor or non-hygienic hand. Poor milking techniques can increase the risk of dairy cows exposed to inflammation and mastitis if they are not done correctly¹⁵.

To reduce the number of bacterial contaminations of milk, it is necessary to pay attention to the cleanliness of the milker and to use sterile milking equipment during milking. Before milking the udder is cleaned with warm water, doing teat dipping before and after milking⁴.

CONCLUSION

Based on this research, can be concluded that there is a significant difference in the number of bacteria milked by machine and conventionally in Medowo, Kediri. The average number of bacteria in Holstein Friesian cow's milk that is milked using machine meets predetermined standards of 3.5×10^5 CFU/mL, while the average number of milk that is milked conventionally is 2.9×10^6 CFU/mL which does not meet the standards of SNI.

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