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THE EFFECTIVENESS OF APPLICATIONS OF BETEL (Piper Betel Linn.) LEAF EXTRACT AND BACTERIOCIN FOR TEAT DIPPING DURING MILKING HANDLING IN INDONESIAN DAIRY FARMING

Iyep Komala[1a], Irma Isnafia Arief[2a]*, Afton Atabany[3a], Lucia Cyrilla ENSD[4a]

Abstract: This study aims to investigate the effectiveness of teat dipping using sirih leaf extract (Piper betle) and bacteria in dairy farms located in Cijeruk Subdistrict, Bogor Regency, West Java. The research employs an experimental design utilizing a Completely Randomized Design (CRD) with four treatments: (1) teat dipping with masofilm (2% iodine) as the positive control, (2) teat dipping with bacteriocin (protein content = 11.17 g mL-1) (P1), (3) teat dipping with betel leaf extract (25% concentration-based) (P2), and (4) teat dipping without any additional treatment, serving as the negative control. Each treatment is replicated three times. The observed parameters include the reduction in somatic cell count (SCC) and total plate count (TPC) in cow's milk. Data were collected through sampling from 12 cows and analyzed using milk quality analysis to assess the conditions of dairy farms in Cijeruk Subdistrict.

Keywords: *Somatic cell count, teat dipping, total plate count*

1. Introduction

Milk, a food source of livestock origin, contains high nutritional value and serves as an essential source of animal protein needed to improve people's quality of life. In 2021, the population of dairy cows in Indonesia was approximately 600,000 heads, with milk production reaching almost 997,000 tons (Statistical Data on Livestock and Animal Health, 2021). However, the population and productivity of dairy cows do not match the level of milk consumption of the Indonesian population, which consists of 268 million people. According to data from the Central Statistics Agency (2021), Indonesia's milk protein needs reached 4.3 million tons, but only 997 thousand tons of fresh milk were produced domestically. Consequently, fresh milk in Indonesia meets only 22% of the national demand, and 78% comes from imports, resulting in a high level of milk consumption. One of the factors that most often causes low milk production is inflammation of the udder in dairy cows, commonly known as mastitis (Wahyuni et al., 2021). Milk consumption in Indonesia remains relatively low compared to other countries, despite milk's numerous benefits for growth. Milk is crucial for cell regeneration, strengthening bones and teeth, supporting physical growth, increasing intelligence, preventing stunting in children, and enhancing body immunity (Belitz et al., 2009; Muehlhoff et al., 2013). Therefore, minimizing the potential for infection and disease in dairy farming is of urgent importance.

Mastitis, an inflammatory disease of the udder caused by microorganisms such as Streptococcus bacteria, often infects dairy cattle in Indonesia and results in significant losses for dairy farms, including the cost of care and treatment, decreased milk production, and decreased milk quality (Surjowardojo, 2011). Livestock productivity declines due to the udder becoming inflamed, hardened, and even bleeding from infections caused by pathogenic microorganisms (Riyanto et al., 2016). Consequently, it is essential to implement disease prevention management through hygienic milking procedures, including teat dipping, at every milking activity.

Teat dipping is a preventive measure aimed at improving hygiene by immersing the nipples in an antiseptic solution to prevent bacteria from entering the nipple canal before and after milking. Mastitis remains the most prevalent bacterial disease on dairy farms, and adult dairy cows require treatment with antimicrobials to prevent it (Pereyra et al., 2015). Povidone-iodine, a chemical antiseptic capable of killing bacteria within 3-5 minutes, is a commonly used antimicrobial in Indonesia for teat dipping. However, this chemical compound has a disadvantage: it can leave chemical residues in milk, leading to contamination (Aprilia et al., 2016). To address bacterial contamination and the incidence of mastitis in dairy cow's milk, alternative solutions such as betel leaf extract and bacteriocin can be employed as substitutes for povidone-iodine in teat dipping to determine milk quality in dairy cows.

As awareness of the adverse effects of chemical products grows, the importance of natural products in health and medicine also increases due to their perceived safety, affordability, and fewer side effects. One such medicinal plant is the betel leaf (Piper betel Linn.), which has essential oil and extract that function as a fungicide and bactericide, exhibiting activity against

Authors information:
[a]Jl. Raya Dramaga, Babakan, Departement of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor, INDONESIA. E-mail: iyepko@apps.ipb.ac.id[1]; isnafia@apps.ipb.ac.id[2]; aftonat@apps.ipb.ac.id[3]; luciacyrilla@apps.ipb.ac.id[4]
*Corresponding Author: isnafia@apps.ipb.ac.id

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various Gram-positive and Gram-negative bacteria. According to Komala (2003), betel leaf extract, using an absolute methanol solvent at concentrations of 50% and 25%, has superior antibacterial power compared to bacitracin 10 U, chloramphenicol 30g, streptomycin 10g, sulfonamides 300 g, and vancomycin 30 g. Based on the antibacterial potency of betel leaf, this study investigates the benefits of betel leaf as a natural antimicrobial, with the expectation that it can be used as an aseptic agent during milking to reduce pathogenic bacterial contamination in cow's milk.

Bacteriocins are peptide compounds produced by lactic acid bacteria that have antimicrobial activity. This antimicrobial compound is non-toxic to humans, easily degraded by proteolytic enzymes, does not harm the intestinal microflora because it is easily digested by digestive enzymes, and is stable to changes in pH and temperature (Hata et al., 2010). Consequently, bacteriocins can be used as biopreservatives in both fresh and processed food products. The use of bacteriocins, such as plantaricin IIA-1A5, as a natural biopreservative containing antimicrobial compounds can damage and kill pathogenic bacteria like S. aureus and E. coli, which often contaminate cow's milk during the process (Arief et al., 2015a). Plantaricin IIA-1A5, isolated from Lactobacillus plantarum IIA-1A5, inhibits the growth of pathogenic bacteria by damaging cell membranes (Arief et al., 2015a; Fatmarani et al., 2018). Therefore, it is necessary to analyze the improvement of milk quality through the application of bacteriocin biopreservatives during the handling of milking in smallholder dairy farms. This study aims to determine the effectiveness of teat dipping with betel leaf extract (Piper betel) and bacteriocin compared to iodine on the quality of dairy cow's milk in Cijeruk farm, Bogor, West Java.

2. Methodology

2.1 Extraction of Betel Leaves

The extraction of betel leaf was performed according to the method proposed by Poeloengan et al. (2006) using ethanol extraction, since this is the extract that has the best effectiveness in killing bacteria that cause mastitis in dairy cows (Poeloengan et al. 2006).

2.2 Bacteriocin Production (Arief et al 2015a)

This study utilizes bacteriocins produced by the Animal Product Technology Laboratory. These bacteriocins are isolated from lactic acid bacteria Lactobacillus plantarum IIA-1A5, which were grown in cheese-whey media. The bacteriocins are in liquid form and constitute antimicrobial peptide compounds (Arief et al., 2015a; Arifin et al., 2020).

2.3 Bioaseptic intervention treatment of betel leaf extract and bacteriocin

The study was conducted on 12 cows during their normal lactation period, specifically from the 3rd to the 5th month. Each cow had two quarters sampled, resulting in a total of 24 quarters used in the research. The dipping treatment stage of the study spanned 22 days. Farmers whose cows were selected as research samples received instructions and were provided with a teat dipper, as well as solutions of iodine, betel leaf extract, and bacteriocin. Teat dipping was carried out by the farmer, who immersed each nipple into a teat dipper containing a liquid of iodine, betel leaf extract, or bacteriocin for 5 seconds per nipple.

The treatments were as follows:

Positive control = teat dipping with masofilm (2% iodine)

Treatment 1 = teat dipping with bacteriocin (protein content = 11.17 g mL-1 based on Soenarno (2019))

Treatment 2 = teat dipping with betel leaf extract (25% concentration based on Komala (2013))

Negative control = teat dipping without any additional treatment

2.4 Sampling

Milk sampling occurred on days 0, 7, 14, and 21. Collection took place in the morning after the cows had been bathed, using an aseptic method that involved cleaning the cow's nipples with 70% alcohol. Pre-labeled test tubes were prepared to prevent errors. The first and second jets of milk were discarded, and the third and subsequent jets were collected in sterile sample tubes, labeled with a code, containing 10-15 ml of milk. The samples were placed in a cool box with an ice pack, maintaining a temperature between 4-7°C. Samples were then transported to the laboratory for immediate analysis.

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2.5 Total Microbial Analysis

The microbiological quality of fresh cow's milk includes an analysis of Total Plate Count (TPC). TPC measurement is performed by taking 25 mL of fresh cow's milk and placing it in 225 mL of Buffered Peptone Water (BPW, Oxoid) solution. Dilutions of 10-4, 10-5, and 10-6 are prepared. Plate Count Agar (PCA, Oxoid) media is poured into a petri dish containing 20 mL of the solution and homogenized, forming a figure-eight pattern. The petri dish is then incubated upside down for approximately 24 hours at 37°C. Aerobic bacterial colonies will appear white. Colony counts are calculated based on the number of eligible colonies (25-250 colonies). Microbial colonies are quantified using the Standard Plate Count (SPC) method (BAM, 2001), applying the following formula.

$$\text{cfu/m} = \frac{\text{N cup}}{(\text{n1} + (0.1 \times \text{n2})) \times \text{d}}$$

Explanation:

N : Number of different colonies in the counting range (25-250 colonies)

n1 : Number of fit plates whose colonies can be counted

n2 :Number of second plates whose colonies be counted

d : First dilution that counts

2.6 Total of Somatic Cell (object-glass Count)

Somatic Cell Count (SCC) testing employs the method outlined by Lukman et al. (2012). The object-glass is cleaned with a 70% alcohol solution and placed on printed paper or a square pattern measuring 1 x 1 cm². The milk sample is first homogenized, and then 0.01 ml of milk is pipetted using a Breed pipette and dripped directly above the 1 cm² box. The milk samples are spread within the 1 cm² square on the object-glass. Breed staining is performed once the milk sample on the glass object is dry. The object-glass is immersed in an alcohol-ether solution for 2 minutes, and subsequently stained by placing it in a methylene blue Löffler solution for 1-2 minutes. The object-glass is then immersed in a 96% alcohol solution for approximately 1 minute to remove any remaining adhering dye. The number of somatic cells is calculated by examining 30 fields of view, totaling the somatic cells, and dividing by the number of fields of view to determine the average number of somatic cells. Once the average number of somatic cells is known, calculations are conducted using the following formula:

Number of Somatic Cells = microscope factor (400 000) x average number of somatic cells

2.7 Physicochemical Characteristics of Fresh Milk

Chemical characteristics, including fat content, lean dry matter (BKTL), specific gravity, lactose, and protein content, were analyzed using a Milkoester (Milkotester LTD; model Eco; serial 35674). A 25 mL fresh milk sample was taken and poured into a cuvette (25 mL). The cuvette was then inserted into the designated slot in the Milkoester. After selecting the desired analysis parameters, the start button was pressed. The Milkoester produced results within 10 minutes and printed them automatically. pH measurements were conducted according to AOAC (2005). A 10 mL milk sample was prepared, and a calibrated pH meter (Hanna) was used, with calibration points at pH 4 and 7. The pH value of the milk was read and recorded.

2.8 Experiments

The study used a Randomized Block Design. The data were processed by analysis of variance (ANOVA). For microbial population analysis, before the analysis of variance, the microbial population data was transformed into a log value of 10. In the case the analysis showed that the treatment had a significant impact on the observed variables, a comparative test was carried out using the Tukey test (Steel et al., 1997).

3. Results & Discussion

3.1 Total of Microbial Contamination in Milk

The microbiological quality of dairy cow's milk serves as a fundamental reference in determining the number of microbes from the bacterial group present. The maximum number of bacteria is a standard requirement for dairy cow's milk. Consequently, environmental factors play a crucial role in the physiological processes of the livestock body, ultimately affecting production capacity and milk quality (Mutaqin et al., 2021). The results of the bacterial counts from each treatment are presented in Table 1.

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Table 1. Total microbial milk samples from dairy farms in Cijeruk

Treatment	Day of Test – (Log cfu/mL)				
	D0	D7	D14	D21	Average
Positive Control (teat dipping iodine)	3.84± 0.09a	4.14 ± 0.09b	4.01 ± 0.02a	3.77 ± 0.27a	3.94 ± 0.31a
Teat dipping bacteriocin	4.00 ± 0.02a	4.14 ± 0.07b	4.29 ± 0.06b	4.27 ± 0.04b	4.01 ± 0.07a
Teat dipping of betel leaf extract	3.98 ± 0.01a	3.28 ± 0.05a	4.44 ± 0.05c	4.40 ± 0.02c	4.02 ± 0.03a
Negative Control	4.0 ± 0.08a	4.41 ± 0.03c	4.52 ± 0.42d	4.37 ± 0.04d	4.22 ± 0.07b

Note: SNI (Indonesian standard) is 5 log cfu/ml or 10^5 cfu/ml(100,000 cfu/ml) Different letters in the same column show a significant difference.

The bacteriocin treatment was effective in maintaining bacterial contamination in fresh milk at the same level as iodine until the 7th day of treatment and was better than the betel leaf extract on the 7th, 14th, and 21st days. The treatment with bacteriocin and betel leaf extract for teat dipping was not significantly different from the treatment using iodine and was even able to reduce the level of bacterial contamination compared to the control (without immersion). Natural compounds such as betel leaf extract and bacteriocin can replace iodine, a chemical for teat dipping in dairy cows in the tropics. An optimal concentration of 25% for betel leaf extract and 1% for bacteriocin (with a protein content of 11.17 gmL-1) can be used as a natural ingredient for teat dipping in dairy farms in the long term.

Iodine functions as an antimicrobial substance that can kill microorganisms, including bacteria, fungi, viruses, protozoa, and spores. The ability of iodine to reduce inflammation is due to its ability to coat the udder with the active substance in the solution, preventing the entry and development of bacteria that cause udder infection (Priono et al., 2016). Iodine's antimicrobial mechanism is caused by its strong oxidizing effect on amino acid groups, nucleotides, and double bonds of unsaturated fatty acids of microorganisms (Noor and Apriasari, 2014). Teat dipping with iodine can reduce inflammation levels because it coats the udder with the active substance in the solution, preventing the entry and development of bacteria that cause udder infection (Priono et al., 2016).

The antibacterial mechanism of bacteriocins and betel leaf extract differs. Bacteriocins are antimicrobial peptides that have bactericidal ability against Gram-positive and Gram-negative bacteria. Bacteriocin plantaricin IIA-1A5 used in this experiment plays a bactericidal role against another bacterial cell, as reported by Arief et al. (2015a). Plantaricin IIA-1A5 displayed a pore-like structure on the surface cells, promoting the release of genetic and proteinaceous materials from the cell, resulting in cell rupture and cell membrane disruption (Arief et al., 2015a). On the other hand, betel leaf extract contains antimicrobial substances such as volatile oils and phenolic components that show antibacterial mechanisms by disrupting cell permeability and causing an effect on unbalanced materials within and outside the cell (Tyagi et al., 2015). Betel leaf extract contains a carboxyl group in the aromatic hydrocarbons of the phenolic compounds that form complexes with extracellular and soluble proteins of bacteria, changing the protein composition that causes bacteria to die (Lubis et al., 2020).

Bacteriocins exhibit antimicrobial activity against foodborne pathogens. Lactic acid bacteria (LAB) are known to produce bacteriocins that display antimicrobial properties against several gram-positive and negative bacteria (Sapatnekar et al., 2010). Lactobacillus plantarum is a bacteriocin-producing strain of LAB known as Plantaricin (Diep et al., 1996; Holo et al., 2001; Maldonado et al., 2003). Bacteriocins generally function by killing bacteria through the formation of pores in the cell membrane, causing the cells to leak and ultimately die (Arief et al., 2015a). Optimal antimicrobial activation of plantaricin PASM1 from Lactobacillus plantarum A-1 ranged from 90% to 100% at a pH range of 5.5 to pH 7 (Gillor et al., 2008). Plantaricin IIA-1A5, belonging to class IIa bacteriocins (Arief et al., 2015a; Mutmainna et al., 2021), can be used as a natural preservative in meat to reduce microbial contamination. Plantaricin IIA-1A5 is also effective as a natural preservative in meat stored at room temperature by inhibiting Escherichia coli and Staphylococcus aureus (Sihombing et al., 2015). Plantarisin IIA-1A5 was found to decrease the total microbe count in fresh milk, and its effective dose for application in dairy smallholder farms is at a concentration of 11.17 g mL^-1 (Soenarno, 2019; Arifin et al., 2020).

Betel leaf contains various elements, such as Si, Cl, Zn, Mg, Ca, and K, which can be used to neutralize acid-base metabolic imbalances in the human body (Periyanayagam et al., 2014). Betel leaf is also rich in metabolites such as volatile oils (safrol, eugenol, eugenol methyl ester, isoeugenol), phenolic components (chavicol, hydroxyl chavicol), hydroxyl

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fatty acids (stearate, palmitic, myristic), and fatty acids (stearic and palmitic), which exhibit antibacterial effects and can be used to treat microbial infections (Bangash et al., 2012). The strong antimicrobial effect of betel leaf is due to the presence of esters, flavonoids, alkaloids, and benzoic acid (Foo et al., 2015). Tannin compounds are also found in betel leaf extract. Tannins are astringents and polyphenols in plants that taste bitter and can bind and precipitate proteins (Subroto, 2006). Tannins can interfere with cell permeability by shrinking their cell walls, which can cause bacterial cells to experience growth disorders or even die (Ajizah, 2004). Extracts of Piper betle Linn exhibit a wide zone of inhibition with minimal inhibitory concentrations against Staphylococcus aureus, Streptococcus pyogenes, Candida albicans, and Trichophyton mentagrophyte (Caburian & Osi, 2010).

The average value of TPC is below the standard set by the National Standards Agency 2009 for the Maximum Limit of Microbial Contamination in Food, which is 1.0 × 106 CFU/mL for TPC. The low number of bacteria in fresh milk is attributed to the practice of cleaning the milking area at least twice a day, before milking in the morning and evening, and washing the nipples before milking. Various microorganisms, with Staphylococcus aureus being the dominant one, including Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uber, cause 90 to 95% of mastitis infections in the dairy industry, with Staph. aureus being the primary mastitis-causing pathogen (Son et al., 2016). Microorganism contamination in milk cannot be separated from sanitation management practices. (Firman, 2010). Additionally, the possibility of contamination due to the nipple opening being open after the milking process is a period that is highly susceptible to infection as bacteria can enter the udder. The process of subclinical mastitis infection starts when microorganisms enter the gland through the nipple opening, which remains open after the milking process. Microorganisms thrive and spread to the alveoli, causing damage to the milk produced. The invasion of microorganisms in the udder and the resulting inflammatory reactions can damage the udder's cells, leading to impaired milk production and decreased quality (Aprilia et al., 2016). In this study, the application of hygiene and sanitation practices on cijeruk farms has been maximized, resulting in no farms exceeding the SNI threshold for TPC number.

3.2 Total Somatic Count Cells (SCC)

A total of 24 quarter samples were used for the total somatic count directly (Breed method). The parameters used in determining subclinical mastitis based on direct SCC calculations are 400,000 cells/ml (Sudarwanto, 1999). The number of somatic cells is related to the mastitis infection (Son et al., 2016). The results of the SCC test are shown in Table 2.

Table 2. Determination of subclinical mastitis based on SCC (n=24)

Treatment	Total Average of SCC day- (cell/ml)			
	H0	H7	H14	H21
Positive Control (teat dipping iodine)	1.375×10^5 a	8.889×10^4 a	1.524×10^5 a	3.098×10^5 a
Teat dipping bacteriocin	8.727×10^4 a	1.429×10^5 a	1.552×10^5 a	1.322×10^5 a
Teat dipping of betel leaf extract	1.377×10^5 a	1.782×10^5 a	5.778×10^4 a	1.277×10^5 a
Negative control	3.556×10^5 a	3.615×10^5 a	7.133×10^5 b	8.502×10^5 b

Note: a = small amount of SCC (< 4 x 105 cells/ml; no subclinical mastitis); b= high number of SCC (more than 400,000 cells/ml; meaning subclinical mastitis.

Based on Table 2, it is evident that teat dipping treatment is much more effective than no treatment. Iodine, bacteriocin, and betel leaf extract were able to keep the total somatic cell count lower and did not exceed the standard, thereby preventing the occurrence of subclinical mastitis. In contrast, without teat dipping, udders of dairy cows experienced subclinical mastitis on days H14 and H21. The effects of mastitis on productivity, reproductive performance, and product quality have been quantified, and diagnostic tools such as SCC testing are used to identify subclinically infected cows (Ruegg, 2017). Somatic cell count (SCC) concentration is a well-established direct indicator of mammary gland infection, i.e., mastitis (Rainard et al., 2008). No cells/mL or (-) indicates that a farm has implemented good management practices but has not implemented a proper mastitis control program.

SCC of 500,000 cells/mL (1+: mild mastitis; 2+: moderate mastitis; 3+: severe mastitis) indicates that one-third of the quarter of cows on the farm suffer from subclinical mastitis (Sudarwanto & Sudarwika, 2008). Based on Table 2, it was found that 87.5% of the total sample of 24 showed SCC values below the standard mastitis incidence, while 12.5% had subclinical mastitis, which occurred in dairy cows without treatment on the 14th and 21st days of the milking period. Fatmawati et al. (2019) state that if the SCC reaches >500,000 cells/mL, it indicates that one-third of a quarter of dairy cows on a farm suffer from subclinical mastitis. According to Sudarwanto and Sudarnika (2006), a somatic

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cell count of <400,000 cells/mL of milk is an indication that a farm has implemented good livestock management practices but has not implemented a comprehensive mastitis control program. Management strategies should be recommended to reduce the spread of contagious pathogens (Ruegg, 2017). The FAO and IDF (2011) provide guidelines on milking practices, stating that the application of teat disinfectant to each teat after milking should be a treatment according to national regulations (Ahmed et al., 2021).

A similar study was conducted by Twomey et al. (2000), who used lactacin 3147, a bacteriocin from Latococcus lactis, as a teat dipping treatment in dairy cows. Teat dipping using lacticin 3147 was found to reduce S. aureus contamination in lactating cows, significantly reducing the risk of mastitis. Consistent with the results of the microbial count in milk, teat dipping treatment using iodine, betel leaf extract, and bacteriocin was proven to prevent the incidence of subclinical mastitis in the udders of dairy cows. The limitations of antimicrobial therapy have been recognized, but the use of antibiotics to treat cows affected with some pathogens remains an important tool for mastitis control (Ruegg, 2017). This is related to the antimicrobial compounds contained in each ingredient of the teat dipping solution, i.e., iodine, betel leaf extract, and bacteriocin, as described in the previous section. Betel leaf extract and bacteriocin were found to be effective and no different from iodine as teat dipping ingredients for dairy cows on smallholder farms.

3.3 Physicochemical Properties of Milk

Fresh milk is a liquid that comes from the udders of healthy and clean cows obtained through proper milking, whose natural content has not been reduced or added to, and has not received any treatment except for cooling. Fresh milk must meet certain requirements to be safe for consumption and use in further processing. Therefore, testing the quality of fresh milk must be continuously carried out for a certain period on dairy farms. The results of testing the quality or physiochemical properties of fresh milk produced from the research are shown in Table 3.

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Table 3. Physicochemical properties/quality of fresh milk

Parameters	Observation Day					Indonesian Standard
	D0	D7	D14	D21	Average	
pH Value						
Positive Control (teat dipping iodine)	6.75 ± 0.05	6.66 ± 0,08	6.72 ± 0.02	6.63 ± 0.03	6.69 ± 0.06	
Teat dipping bacteriocin	6.62 ± 0.03	6.65 ± 0,05	6.62 ± 0.03	6.68 ± 0.03	6.64 ± 0.03	
Teat dipping of betel leaf extract	6.63 ± 0.02	6.70 ± 0.05	6.62 ± 0.02	6.73 ± 0.08	6.67 ± 0.12	
Negative Control	6.67 ± 0.06	6.67 ± 0.06	6.67 ± 0.06	6.65 ± 0.09	6.66 ± 0.06	
Average	6,65 ± 0.04	6.67 ± 0.06	6.66 ± 0.03	6.67 ± 0.06	6.66 ± 0.06	
Protein Level (%)						≥ 2.80
Positive control (teat dipping iodine)	3.10 ± 0.10	3.08 ± 0.08	3.05 ± 0.13	3.03 ± 0.07	3.06 ± 0.09	
Teat dipping bacteriocin	3.11 ± 0.15	3.13 ± 0.24	3.11 ± 0.15	3.21 ± 0.05	3.14 ± 0.15	
Teat dipping of betel leaf extract	3.20 ± 0.07	3.13 ± 0.05	3.21 ± 0.07	3.25 ± 0.25	3.20 ± 0.11	
Negative control	3.10 ± 0.10	3.10 ± 0.10	3.10 ± 0.10	3.15 ± 0.10	3.11 ± 0.10	
Average	3.13 ± 0.11	3.11 ± 0.12	3.12 ± 0.11	3.16 ± 0.12	3.13 ± 0.11	
Fat Level (%)						≥3.0
Positive control (teat dipping iodine)	2.00 ± 0.30	2.5 ± 0.40	2.61 ± 0.65	3.70 ± 0.22	2.70 ± 0.39 a	
Teat dipping bacteriocin	2.60 ± 0.40	2.1 ± 0.40	2.66 ± 0.45	3.80 ± 0.21	2.85 ± 0.37 a	
Teat dipping of betel leaf extract	2.40 ± 0.90	3.5 ± 0.25	2.40 ± 0.90	2.70 ± 0.11	2.77 ± 0.14 a	
Negative control	1.61 ± 0.24	1.5 ± 0.20	1.60 ± 0.20	0.90 ± 0.40	1.42 ± 0.28 b	
Average	2.15 ± 0.46	2.4 ± 0.31	2.32 ± 0.55	2.78 ± 0.24	2.44 ± 0.30	
Lactose Level (%)						≥4.0
Positive control (teat dipping iodine)	4.60 ± 0.10	4.60 ± 0.10	4.50 ± 0.10	4.50 ± 0.10	4.61 ± 0.14 b	
Teat dipping bacteriocin	4.70 ± 0.20	4.70 ± 0.30	4.70 ± 0.20	4.80 ± 0.10	4.75 ± 0.23 ab	
Teat dipping of betel leaf extract	4.80 ± 0.10	4.75 ± 0.08	4.80 ± 0.10	5.20 ± 0.05	4.93 ± 0.20 a	
Negative control	4.60 ± 0.10	4.60 ± 0.20	4.60 ± 0.10	4.70 ± 0.05	4.68 ± 0.15 ab	
Average	4.68 ± 0.13	4.66 ± 0.17	4.65 ± 0.13	4.80 ± 0.75	4.74 ± 0.18	
Solid Non Fat (%)						≥7.8
Positive control (teat dipping iodine)	8.40 ± 0.2	8.4 ± 0.3	8.3 ± 0.3	8.3 ± 0.1	8.41 ± 0.28	
Teat dipping bacteriocin	8.60 ± 0.4	8.6 ± 0.6	8.6 ± 0.4	8.8 ± 0.2	8.7 ± 0.4	
Teat dipping of betel leaf extract	8.80 ± 0.2	8.7 ± 0.1	8.8 ± 0.2	8.9 ± 0.6	8.85 ± 0.30	
Negative control	8.60 ± 0.2	8.5 ± 0.3	8.6 ± 0.2	8.65 ± 0.05	8.6 ± 0.2	
Average	8.60 ± 0.3	8.6 ± 0.3	8.6 ± 0.3	8.66 ± 0.23	8.64 ± 0.3	

Note: different letters for the same column in each parameter indicate a significant difference ($p<0.05$)

Based on the results of the statistical analysis, it was found that teat dipping treatment and milking time had no significant effect on the pH value, protein content, and solid non-fat content of the fresh milk produced. There was no interaction between the factors of teat dipping treatment and milking time. However, the teat dipping treatment had a P-value < 5%, which indicates that the treatment affected the fat content. In Tukey's test, the control treatment without teat dipping had a lower effect than the other treatments on fat at a significant level of 5%.

Teat dipping treatment and milking time had a significant effect on lactose content (P-value < 5%). In Tukey's test, fresh milk treated with teat dipping iodine had lower lactose content than fresh milk treated with teat dipping betel leaf

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extract. This may be due to the fat content in the betel leaf extract that is included during the milking process.

The requirements for fresh milk include a minimum fat content of 3.0%, solid non-fat (SNF) of at least 7.8%, and protein content of at least 2.8% (National Standardization Agency, 2011). The composition of milk varies depending on several factors. For FH cows, the percentages of water, protein, fat, lactose, ash, and BK are 88.01%, 3.15%, 3.45%, 4.65%, 0.68%, and 11.57%, respectively (Sudono et al., 2003).

The results showed that the pH value, protein content, lactose content, and solid non-fat value of freshly milked milk had values according to Indonesian national standards. However, the fat content of fresh milk in all treatments was lower than the Indonesian national standard due to the feed used, which was mostly forage and less concentrated, resulting in a low fat content. Moreover, consumers also prefer low-fat milk. Dairy farmers provided forage at an average of 47.32 kg head-1 day-1, with an average milk production of 11.29 L head-1 day-1. The feeding of forage based on body weight is estimated only based on the body shape and experience of the dairy farmer. Farmers give concentrate, which has a crude protein content of 14%, with an average of 9.99 kg head-1 day-1. However, the crude protein content of the concentrate fed to cows is lower than the standard, which should contain 19.04% crude protein. Improving the quality of concentrate feed can improve the milk productivity of lactating cows, by feeding forage and concentrate that are adjusted to the live weight of lactating cows. Therefore, the fat content of milk sourced from concentrates is lower than 3.00%.

The effectiveness of various treatments on fresh milk has been studied extensively, with researchers exploring different approaches. For example, a study by Wahyuningtyas et al (2021) found that teat dipping before milking reduced the number of Escherichia coli bacteria and total microbes in milk without compromising its quality (as measured by Indonesian standards). Additionally, researchers have investigated the use of a bio preservative called Plantaricin IIA-1A5, which has been shown to preserve milk quality according to SNI standards and reduce the presence of pathogenic bacteria during storage at room temperature and in cold temperatures (Soenarno et al 2020; Arief et al 2021). These findings demonstrate that Plantaricin IIA-1A5 can be an effective bio preservative or bio aseptic for fresh milk.

4. Conclusion

The teat dipping treatment applied during the milking process for 21 days at a dairy farm in Cijeruk village, Bogor-West Java-Indonesia was successful in improving the microbiological quality of fresh cow's milk and reducing the incidence of subclinical mastitis in cow udders. Teat dipping using natural ingredients such as betel leaf extract and bacteriocin can replace chemical iodine. The physicochemical quality of freshly milked cow's milk was not affected by the teat dipping treatment. Therefore, teat dipping using betel leaf extract and bacteriocin is highly recommended for the management of milking in dairy farms. The recommended concentration for bacteriocin is a liquid with a protein content of 11.17 g/mL, while for betel leaf extract, a 25% concentration is recommended. The practical application of teat dipping is conducted after the milking process for five seconds on each nipple

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