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Study of quality of cows milk treated bay leaf teat dipping in Tegal Mandiri Farmer

Kajian kualitas susu sapi dengan perlakuan celup puting larutan daun salam di Kelompok tani Tegal Mandiri

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ABSTRACT

The aim of this study was to examine the quality of milk including microbiological, chemical properties and detection of subclinical mastitis resulting from the application of bay leaf solution as a natural antiseptic. Application of bay leaf solution was conducted on dairy cows at Tegal Mandiri Farmer Group for 28 days. The parameters observed include microbiological and chemical quality including Total Plate Count (TPC), *Staphylococcus aureus* contamination, *Escherichia coli* contamination, fat, protein, lactose content, density and freezing point. Subclinical mastitis was detected using the Draminski Mastitis Detector to see the electrical resistance value of the milk. The results showed that the milk resulting from the application of teat dipping with bay leaf solution had microbiological qualities including Total Plate Count and *S. aureus* population which met the standards with a TPC population of 4.70 log CFU/ml and *S. aureus* 0.91 log CFU/mL, but the *E. coli* population did not meet the standards. Application of teat dipping with bay leaf solution can reduce the population of *S. aureus*. Chemical quality in the form of fat content, protein, specific gravity, lactose and freezing point is in accordance with standards. An electrical resistance value of >300 indicates that the milk is of high quality, healthy and has a low incidence of mastitis.

Kata kunci:
Daun salam
Susu
Mastitis subklinis
Celup puting

ABSTRAK

Penelitian ini memiliki tujuan untuk mengkaji kualitas susu meliputi sifat mikrobiologi, kimia, dan deteksi mastitis subklinis hasil aplikasi celup puting larutan daun salam sebagai antiseptik alami. Aplikasi celup puting larutan daun salam dilakukan pada sapi perah di Kelompok tani Tegal Mandiri selama 28 hari. Parameter yang diamati meliputi kualitas mikrobiologi dan kimia diantaranya Total Plate Count (TPC), cemaran Staphylococcus aureus, cemaran Escherichia coli, kandungan lemak, protein, laktosa, berat jenis, dan titik beku. Dilakukan deteksi mastitis subklinis menggunakan Draminski Mastitis Detector untuk melihat nilai hambatan listrik susu. Hasil menunjukkan bahwa susu hasil aplikasi celup puting larutan daun salam memiliki kualitas mikrobiologi diantaranya Total Plate Count dan populasi S. aureus yang memenuhi standar dengan populasi TPC 4.70 log CFU/ml dan S. aureus 0.91 log CFU/ml, namun populasi Escherichia coli tidak sesuai standar. Aplikasi celup puting larutan daun salam nyata dapat menurunkan populasi S. aureus. Kualitas kimia berupa kadar lemak, protein, berat jenis, laktosa dan titik beku sesuai dengan standar. Nilai hambatan listrik >300 menunjukkan bahwa susu berkualitas tinggi, sehat dan memiliki insiden mastitis yang rendah.

INTRODUCTION

In developing countries like Indonesia, mastitis is a disease with a high prevalence on dairy farms. The prevalence of subclinical mastitis even reached 82.54% in West Java and 87% in East Java. The average prevalence of mastitis in Indonesia is higher than in China and Ethiopia (Nuraini, Andityas, Sukon, & Phuektes, 2023). This is a major problem, especially in subclinical mastitis conditions which does not show symptoms of inflammation. The impact of mastitis is massive, such as reducing milk production in large quantities and causing a significant decrease in economic value, especially for smallholder farmers. On the other hand, milk production in Indonesia is only able to meet around 20% of the total national demand, so the rest must be imported (Wulandari & Bowo, 2019).

Mastitis is inflammation of the mammary gland predominantly caused by intramammary invasion of pathogens in the udder (Martin, Barkema, Brito, Narayana, & Miglior, 2018). Mastitis is most commonly caused by bacterial infections. The prevalent mastitis-causing bacteria are *Streptococcus* species (*S. aureus, S. intermidus, S. hycus)*, and *Escherichia coli* (Fesseha, Mathewos, Aliye, & Wolde, 2021). The most common bacteria that cause mastitis are *S. aureus, S. agalactiae*, and *E. coli*. Beside affecting the quantity of milk, the condition of a cow suffering from mastitis also affects the quality of the milk produced. Microbial contamination can cause deterioration in milk. This deterioration can make unwanted protein coagulation, taste alterations, and a rise in the amount of free fatty acids and amino (Velázquez-Ordoñez et al., 2019). Moreover, the quality of milk from traditional dairy farmers is generally still below standard, so the selling price at the cooperative level and the milk processing industry is still low.

Disease control is one of the important components in the livestock business that must be carried out. The milking process is a critical point in the spread of mastitis agents that hygienic milking procedures need to be implemented. One of the sanitation procedures during milking is teat dipping. Teat dipping using antiseptic aims to prevent bacteria from entering the udder through the teat hole. Solutions commonly used for teat dipping include chlorhexidine, chlorine, and iodine (Ózsvári & Ivanyos, 2022). The most frequently used solution is iodine. However, the use of this chemical solution may leave residues in milk. Iodine residue in milk can risk consumers health if consumed over a long period of time. Natural ingredients are an alternative to replace the role of iodine as an antiseptic. One of natural ingredient that has potential is bay leaves. Bay leaves have antibacterial activity due to the presence of alkaloids, flavonoids, saponins, tannins and triterpenoids (Kilis, Karauwan, Sambou, & K, 2020). Furthermore, bay leaves are affordable, safe, and available in many areas of Indonesia. The technique for making bay leaf solution for teat dipping is also simple to make it easier for farmers. The effect of using bay leaf solution can be seen through the Draminski Mastitis Detector subclinical mastitis detection test, the presence of *S. aureus, E. coli* bacteria, Total Plate Count, and testing the chemical properties of milk, therefore the aim of this research was to examine the quality of milk including microbiological and chemical properties, and detection of subclinical mastitis as a result of application of bay leaf solution as a natural antiseptic.

MATERIALS DAN METHODS

The research was carried out using 10 Friesian Holstein dairy cows in their first lactation in the Tegal Mandiri Farming Group, Cisarua District, Bogor Regency. The cows were classified as 5 heads without bay leaf dipping treatment, and 5 heads with bay leaf treatment. Independent t-test was used to compare the means of the two groups. The materials used include bay leaves, distilled water, Plate Count Agar (PCA), Eosin Methylene Blue Agar (EMBA), Buffer Peptone Agar (BPA), Buffer Peptone Water (BPW) 0.1%. The equipment used include Draminski Mastitis Detector, Milko tester, Cooling box, Teat Dipper, Incubator. Detection of subclinical mastitis was carried out at the dairy farm location, microbiological tests and milk quality tests were carried out at the livestock quality assurance laboratory, Department of Animal Husbandry, Bogor Agricultural Development Polytechnic.

Preparation of Bay Leaf Decoction

Bay leaves were washed until clean with running water and then dried. Three hundred gram dried bay leaves were chopped and boiled using 1000 ml distilled water with a bay leaf concentration of 30% for 15 min at 100 ^{o}C. The solution was filtered, cooled, and stored in a Scott bottle (Modification of Atam, Widjaya, Permana, Akhdiat, & Christi, 2020).

Application of Teat Dipping

The bay leaf solution was put into a teat dipper bottle. After the milking process was complete, each teat was dipped in the bay leaf solution for ± 30 seconds. Teats were allowed to air-dry naturally. Teat dipping was carried out for 28 days.

Subclinical Mastitis Detection using Draminski Mastitis Detector

The electrical resistance test was carried out using the Draminski Mastitis Detector 4QMAST tool referring to the tool's instruction manual. The test was carried out directly using first squirts of milk. The device was turned on by pressing the "on" button. The cup on the device was positioned under the teat until it filled with milk to the line of the cup. Next, "ok" button was pressed, then wait until the electrical resistance value appears on the screen. Before running the next quarter. The milk was discarded into bucket, and "on" button was pressed. The remaining quarter were processed as before.

Milk Sampling

Milk samples were taken aseptically, the teats were cleaned first. The squirts were taken as much as 100 ml into a Scoot bottle, labeled and stored in a cool box containing ice packs at a temperature of 4-10^{o}C for further microbiological testing.

Total Plate Count Assay

Twenty five ml of milk sample was put into 225 ml of 0.1% BPW solution. One 1 ml of suspension was taken to be diluted into 9 ml of BPW, then the dilution was carried out again to 10^{-5}. As much as 1 ml of suspension from each dilution was into a sterile petri dish in duplicate. 20 ml of Plate Count Agar which had been cooled to a temperature of 40^{o}C was poured into each petri dish containing the suspension. Incubation was carried out at 37^{o}C for 48 hours in an inverted position. The number of growing colonies was calculated on colony counter (Erikatriayu, Sinaga, & Rusdhi 2023).

Staphylococcus aureus Assay

A total of 25 ml of milk sample was put into 225 ml of 0.1% BPW solution. Then 1 ml of suspension was taken and diluted into 9 ml of BPW, then the dilution was carried out again to 10^{-3}. A total of 20 ml of Bair Parker Agar which had been added with egg yolk and tellurite emulsion was poured and allowed to solidify in a petri dish. Aseptically, 1 ml of the suspension was inoculated in petri dish then spread evenly on the surface of the media and allowed to absorb. Incubation was carried out at 37^{o}C for 45-48 hours in an inverted position. The number of growing colonies was calculated on colony counter (Sandra Tallent, Hait, Bennett, & Lancette, 2016).

Escherichia coli enumeration

A total of 25 ml of milk sample was put into 225 ml of 0.1% BPW solution. As much as 1 ml of suspension was taken to be diluted into 9 ml of BPW, then the dilution was carried out again to 10^{-5}. 1 ml of suspension from each dilution was inoculated in a sterile petri dish in duplicate. A total of 20 ml of Eosin Methylene Blue Agar which had been cooled to a temperature of 40^{o}C was poured into each petri dish containing the suspension. Incubation was carried out at 37^{o}C for 24 hours in an inverted position. The number of growing colonies was calculated on colony counter (Feng, Weagant, & Grant 2020)

RESULTS AND DISCUSSION

Microbiological Quality of Milk

The microbiological quality of cow's milk in the Tegal Mandiri Farmers Group can be evaluated through the microbial population (Total Plate Count), *E. coli and S. aureus*. Total Plate Count (TPC) shows the microbiological quality of milk and determines the suitability of milk for consumption. The presence of *E. coli* and *S. aureus* bacteria also determines the quality of milk, especially these two bacteria cause subclinical mastitis. The data in Table 1 shows the bacterial population in milk from cows that treated teat dipping using bay leaf solution and those that did not treat teat dipping on day 0 and day 28.

Based on the test results, the *S. aureus* population decreased significantly ($P<0.05$) after teat dipping treatment using bay leaf solution for 28 days, while the TPC and *E. coli* values were not significantly different before and after treatment.

Table 1. Microbiological properties of cow's milk from Tegal Mandiri Farmers Group

		Population (log CFU/ml)	
		P0	P1
Day-0	TPC	5.02±0.46	4.99±0.51
	E. coli	2.66±0.51	2.44±0.62
	S. aureus	1.57±0.15	2.02±0.44[a]
Day-28	TPC	5.13±0.69	4.70±0.27
	E. coli	2.28±0.18	2.34±0.62
	S. aureus	2.05±0.50	0.91±0.31[b]

P0= without teat dipping; P1=Teat dipping using bay leaf solution; [abc] Different superscripts in the same column indicate significant differences (P<0,05)

This shows that teat dipping using bay leaf solution is effective in reducing *S. aureus* bacterial contamination. This is in line with research of Algabri, Doro, Abadi, Shiba, & Salem (2018) which shows that the methanol extract of bay leaves has antimicrobial activity with an inhibition zone for *S. aureus* of 18 mm, but there is no inhibition for *E.coli.* This ability comes from the phytochemical content in bay leaves such as alkaloids, flavonoids, tannins, saponins and triterpenoids (Kilis et al., 2020). Flavonoids have the ability to interact with bacterial DNA by damaging the permeability of bacterial cell walls. Saponins are also able to reduce the surface tension of cell walls by denaturing proteins and damaging the cell cytoplasm. This disturbed condition of the bacterial cell wall is an opportunity for tannin to enter and coagulate the protoplasm of the *S. aureus* bacterial cell, resulting in cell growth being inhibited and even dying (Aprilia, Santoso, & Harjanti, 2016). The difference in the effectiveness of bay leaf teat dipping on populations of *S. aureus* and *E. coli* might be due to structural differences in the two bacteria. *S. aureus* is a Gram positive bacteria, while *E. coli* is a Gram negative bacteria. Gram positive bacteria have a single layer of their plasma membrane, while Gram negative bacteria have a more complex layer (an inner membrane and an outer membrane). Due to their distinctive structure, Gram-negative bacteria are more resistant than Gram-positive bacteria. The outer membrane of Gram-negative bacteria is the main reason for resistance to a wide range of antibiotics (Breijyeh & Karaman, 2020).

The TPC value of milk, whether from cows treated with teat dipping or without treatment, still meets the quality requirements for fresh milk according to Indonesian National Standard 3141.1 of 2011, which set a maximum population of $1x10^6$. Even though the t test results showed no significant difference, descriptively, the total microbes in cow's milk resulting from the application of teat dipping were lower than those that did not apply teat dipping. Based on Indonesian National Standard 3141.1 of 2011, *S. aureus* contamination in fresh milk is a maximum of $1x10^2$ CFU/ml, so the milk *S. aureus* population in this study still meets quality requirements. For *E. coli* contamination, the existing population exceeding Indonesian National Standard 01-6366-2000 which requires the test results for *E. coli* to be negative. This can be affected by sanitation of the cage and environment, hygiene of the farmer, cleanliness of the milking equipment, and conditions during milking. *E. coli* bacteria originate from the intestines and are found in cow feces. Some strains of this bacteria are harmless, but many can cause illnesses such as diarrhea. For this reason, Good Farming Practices (GFP) must be implemented in full. Applying teat dipping after milking is part of disinfection with the aim of killing bacteria on the teat skin and preventing bacteria from the environment from entering the udder through the teat hole. This procedure is important to prevent high levels of bacterial contamination in milk (Hartanto, Harjanti, Prayitno, Restitrisnani, & Prima, 2021).

Chemical Quality of Milk

Data on the chemical quality of control cow's milk and the results of the teat dipping treatment with bay leaf solution can be seen in Table 2.

Based on the test results, the chemical quality of cow's milk that was treated with teat dipping was not significantly different from cow's milk that was not treated with teat dipping. Both have fat content ranging from 3.29 to 4.38%, where this value meets Indonesian National Standard 3141.1: 2011, which set a minimum of 3%. The protein content of milk in this study ranged from 2.90 to 3.26%, in accordance with the minimum standard of 2.8%. The specific gravity of the milk samples ranged from 1,026 to 1,028 g/ml. SNI 3141.1: 2011 requires a minimum specific gravity of 1.027 milk, but according to Burke et al., (2018) dan FAO, The specific gravity of milk ranges from 1,026 to 1,034 g/cm^3. Milk in this study had

Table 2. Chemical properties of cow's milk from Tegal Mandiri Farmers Group

		Chemical properties	
		P0	P1
Day-0	Fat (%)	4.38±0.35[a]	4.38±0.31[a]
	Protein (%)	2.90±0.22	2.93±0.32
	Specific gravity (g/ml)	1.026±0.00	1.028±0.00
	Lactose (%)	4.36±0.33	4.14±0.48
	Freezing point (°C)	-0.530±0.00	-0.538±0.02
Day-28	Fat (%)	3.29±0.50[b]	3.67±0.23[b]
	Protein (%)	2.95±0.43	3.26±0.73
	Specific gravity (g/ml)	1.026±0.00	1.026±0.00
	Lactose (%)	4.27±0.40	4.42±0.73
	Freezing point (°C)	-0.533±0.00	-0.530±0.00

P0= without teat dipping; P1=Teat dipping using bay leaf solution; [abc] Different superscripts in the same column indicate significant differences (P<0,05)

lactose levels ranging from 4.14 to 4.42%, this value is in line with Burke et al., (2018) which states that milk lactose levels vary between 3.6 to 5.5%. Study of Subagyo et al., (2023) also found that the lactose content in cow's milk was 4.54%. Milk in this study had a freezing point of -0.53 to -0.538 °C, which is within the range of freezing point criteria set in SNI 3141.1: 2011, namely -0.520 to -0.560 °C.

According to the chemical quality, the milk samples meet good quality and comply with standards so it can be concluded that the teat dipping treatment using bay leaf solution does not have a bad impact on the chemical quality of the milk, in fact it still produces milk of good chemical quality. Based on observation, there was a decrease in milk fat content on day 28 compared to day 0, both in milk from cows treated with teat dipping and not. This shows that the decline was not caused by the teat dipping treatment but might be caused by other factors such as feed, especially the forage. The quantity and quality of forage determines the composition of the milk produced, especially fat. Providing high amounts of forage will increase the fat content, supported by the statement of Suhendra et al., (2015) that Neutral Detergent Fiber (NDF) which comes from forage affects milk fat content. High NDF content can produce high levels of acetic acid. Acetic acid will be converted into fatty acids which then enter the secretory cells of the udder for milk fat synthesis. Besides feed, according to pise, Sudarwanto, Sovianna, & Pisestyani (2020) Milk fat content is also affected by genetics, rearing methods, climate, lactation period and animal health. Changes in environmental temperature and different health conditions on days 0 and 28 can cause differences in milk fat content.

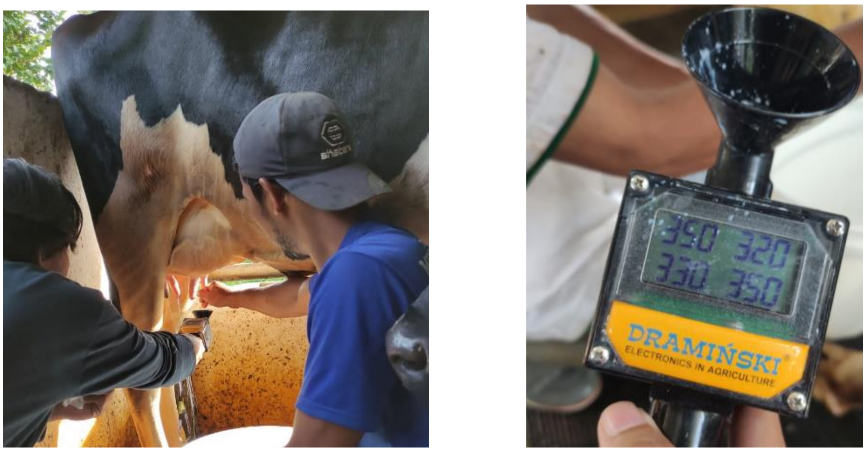
Subclinical Mastitis Detection

Detection of Subclinical Mastitis using the Draminski Mastitis Detector was carried out on days 0 and 28 for cows treated with teat dipping or not (Figure 1). Observations were made by assessment of the electrical resistance value of the milk. The principle of Draminski Mastitis Detector is by measuring changes in electrical resistance caused by changes in electrolyte levels in milk. An increase in milk electrolyte levels occurs in the udder of cows infected with mastitis, the more severe the cells are damaged due to mastitis, the more electrolytes contained (Pisestyani, Permana, Basri, Lukman, & Sudarwanto, 2023; Siddiquee et al., 2013).

According to Galfi et al. (2015), Infection of udder tissue changes the composition of milk, increasing the electrical conductivity of the milk and lowering the electrical resistance of the milk. The electrical conductivity value is determined by the concentration of anions and cations in milk. The concentration of sodium and chloride ions increases in infected milk and increases electrical conductivity. The electrical conductivity of milk has a positive correlation with somatic cells. The same thing was also confirmed by atto, Radiati, Mahdi, & Evanuarini (2021), in udder tissue affected by mastitis, lactose and potassium concentrations decrease, while sodium and chloride concentrations increase. Increased concentrations of sodium and chloride ions in milk originating from infected udders increase the electrical conductivity of the milk.

The electrical resistance values of milk in this study are presented in Table 3 and the results are compared with the interpretation of the draminski mastitis detector results in Table 4.

The electrical resistance values of milk in this study ranged from 304 to 342 units. Based on the interpretation results, the value obtained



(a) (b)

Figure 1. Testing using Draminski Mastitis Detector (a), Draminski Mastitis Detector tool (b)

Table 3. Electrical resistance of cow's milk from Tegal Mandiri Farmers Group

		Electrical resistance	
		P0	P1
Day-0	Quarter I	330±14.14	304±27.01[a]
	Quarter II	327.5±22.17	316±19.49[a]
	Quarter III	332.5±9.57	328±08.36
	Quarter IV	330±11.54	324±19.49[a]
Day-28	Quarter I	322.5±26.29	328±22.08[b]
	Quarter II	342.5±15.00	334±18.16[b]
	Quarter III	335±26.45	340±15.81
	Quarter IV	337.5±25.00	340±14.14[b]

P0= without teat dipping; P1=Teat dipping using bay leaf solution; [abc] Different superscripts in the same column indicate significant differences (P<0,05)

Table 4. Interpretation of draminski mastitis detector results

Unit	Interpretation
>300	High quality and healthy milk samples. The incidence of subclinical mastitis of values above 300 units is very low
250– 300	The incidence of subclinical mastitis increases with decreasing electrical resistance values
<250	Subclinical mastitis progresses to clinical status. Characterized by somatic cells increasing from 1 to hundreds of millions

is >300 units, meaning the milk sample is high quality and healthy. The incidence of subclinical mastitis of values above 300 units is very low. Based on the test results, there was a significant increase in the electrical resistance value ($P<0.05$) on the 28th day for quarters I, II, and IV. Based on the overall value, the numbers show a tendency to increase the electrical resistance value in milk obtained from cows treated with bay leaf solution teat dipping. These results are in line with the microbiological profile of cow's milk resulting from the application of teat dipping with bay leaf solution which shows lower contamination compared to cows that were not treated with teat dipping.

CONCLUSIONS

The application of teat dipping using bay leaf solution to dairy cows produces milk with microbiological quality including the Total Plate Count and *S. aureus* population meeting standards, even teat dipping with bay leaf solution can reduce the *S. aureus* population, but the *E. coli* population does not meet the standards; chemical quality such as fat content, protein, specific gravity, lactose and freezing point met the standards; and an electrical resistance value of >300 which indicates that the milk is of high quality, healthy and has a low incidence of mastitis.

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