Application of Infrared Thermography as a Determinant of Sub-Clinical Mastitis in Sapera Dairy Goats

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(received 04-06-2022; revised 22-06-2022; accepted 23-06-2022)

ABSTRAK

Pamungkas FA, Purwanto BP, Manalu W, Yani A, Sianturi RG. Penggunaan termografi inframerah sebagai penentu mastitis sub-klinis pada kambing perah Sapera. JITV 27(2):65-73. DOI: http://dx.doi.org/10.14334/jitv.v27i2.3059.

Penelitian ini mengevaluasi potensi penggunaan hasil pengindraan termografi inframerah (IRT) dibandingkan dengan somatic sell count (SCC) dan tes reagen mastitis dari IPB University (IPB-1) pada deteksi infeksi mastitis sub-klinis kambing perah Sapera. Sebanyak 8 ekor kambing perah sapera dengan rataan berat badan 35-40 kg dievaluasi selama masa laktasi. Parameter yang diobservasi termasuk produksi susu, karakteristik psikokimia, penginderaan SCC, IPB-1 dan IRT putting. Data yang diperoleh kemudian dianalisis mengikuti prosedur MIXED dan CORR dari SAS. Hasil penelitian menunjukan bahwa karakteristik psikokimia susu (lemak; non-fat solid; laktosa; protein; titik beku; pH), SCC dan IRT berbeda (p<0,05), khususnya hasil mastitis antara normal dan +3. Rataan produksi susu dengan normal hingga skore +2 mastitis selama laktasi sebesar 1,281±253 ml/hari, sementara pada hasil +3 mastitis adalah sebesar 957±250 ml/hari. Korelasi ditemukan pada ambing kanan dan kiri ini merupakan bukti penting yang menunjukan bahwa mengkombinasikan hasil evaluasi parameter dengan metode IRT dengan SCC dan IPB1-SCC (r=0.70-0.74), IPB1-IRT (r=0.70-0.71), dan SCC-IRT (r=0.62-0.65). Hal ini merupakan bukti penting yang menunjukan bahwa mengkombinasikan parameter hasil tes IRT dengan SCC dan IPB-1 dapat bermanfaat untuk skrining mastitis subklinis pada kambing perah.

Kata Kunci: Kambing, Inframerah, Mastitis, Sel Somatik, Termografi

ABSTRACT

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Application of infrared thermography (IRT) sensing results versus somatic cell count (SCC) and mastitis test reagent from Bogor Agricultural University (IPB-1) was evaluated in this study for infection detection in dairy goats with subclinical mastitis. Eight Sapera dairy goats with a 35-40 kg live weight were evaluated throughout their lactation. The parameters observed including milk production, physicochemical characteristics, SCC, IPB1, and IRT sensing in the udder. The collected data were analysed using MIXED and CORR procedures from SAS. Results showed that the physicochemical characteristic of milk (fat, non-fat solids, lactose, protein, freezing point, pH), SCC and IRT were significantly different (P<0.05), especially the test results for mastitis between normal and +3. The average production of goat milk with a normal until +2 mastitis test score during lactation was 1.281±253 ml/day, while a mastitis test score of +3 was 957±250 ml/day. A positive correlation was found in both the left and right udder of IPB1-SCC $(r=0.70-0.74)$, IPB1-IRT $(r=0.70-0.71)$, and SCC-IRT $(r=0.62-0.65)$. This is substantial evidence that combining IRT results with SCC and IPB1 parameters can be valuable for screening subclinical mastitis in dairy goats.

Key Words: Goat, Infrared, Mastitis, Somatic Cells, Thermography

INTRODUCTION

Mastitis is a term used to describe inflammation of the mammary gland's parenchymal tissue, characterised by physical, chemical, and microbiological changes in the milk produced (Radostits et al 2007). In terms of livestock production, mastitis results in a decrease in milk production, changes in milk quality, an increase in medical costs, labour, and the number of livestock released (Saleh and Faye 2011). Mastitis is divided into clinical, subclinical, and chronic mastitis. Clinical mastitis is derived from the presence of inflammatory signs (such as redness, heat, swelling, pain, and loss of udder function); subclinical mastitis is characterised by changes in milk composition without signs of severe inflammation of the udder. At the same time, chronic mastitis is characterised by an inflammatory process that can continue from one lactation to the next (Awale et al 2012).

Clinical mastitis causes economic losses due to decreased or discarded milk production. However, subclinical mastitis causes a more significant loss because the continued presence of microorganisms in the mucosa during one or several lactation periods causes a progressive loss of epithelial secretion, thereby reducing milk production (Srivastava et al 2015). Due to this impact, many studies have been directed toward diagnosing and detecting mastitis, including the California Mastitis Test (CMT), Somatic Cell Count (SCC), and enzymatic analysis (Viguier et al 2009). So far, milk somatic cell count has been used as an indicator of udder health. It has been included in selection indexes of different countries to reduce the susceptibility to mastitis (Weigel & Shook, 2018). Somatic cells are epithelial cells that come out of the lining of the mammary glands to secrete milk. Their number will increase due to the presence of pathogens as indicators of infection in milk (Madouasse et al 2012). SCC values lower than 1×10^5 cells mL⁻¹ of milk indicates good milk quality, whereas milk infected with bacteria can cause an increase in SCC to above 1×10^6 cells mL^{-1} (Bytyqi et al 2010). However, this technique is subjective, time-consuming and labour-intensive, and less accurate for the detection of early signs of disease and has not been scientifically validated for the detection of clinical mastitis, especially in automated milking systems (Hovinen et al 2008) so that a detection method is needed without having to do milking but can detect early signs of mastitis before the physical, chemical, and microbiological changes in the milk produced.

Infrared thermography (IRT) is a non-invasive sensing method used to measure heat transfer and blood flow changes by detecting changes in body temperature (Nääs et al 2014). Kunc et al (2007) reported that the udder affected by mastitis had a high temperature even before clinical symptoms. Recently, there has been speculation regarding the application of infrared thermography (IRT) to obtain udder surface temperature and use it as a tool for mastitis diagnosis (Berry *et al.* 2003). Based on this background, this study aimed to examine the use of non-invasive and non-milking IRT methods in the early detection of mastitis in dairy goats.

MATERIALS AND METHODS

This research was conducted at the Indonesian Research Institute for Animal Production (IRIAP), located at an altitude of 450 to 500 m above sea level with the potential for rainfall between 3500 to 4000 mm year-1 . The air temperature in the cage ranged from 20.81-31.59 °C with a relative humidity of 47.19-99.82 % and a wind speed of 1.18-2.02 m/s. The use of experimental animals has obtained approval from Komisi Kesejahteraan Hewan Balitbangtan (KKHB), Ministry of Agriculture of the Republic of Indonesia, with registration number: Balitbangtan/Balitnak/ Rm/04/2019.

Animals

Eight Sapera dairy goats with a 35-40 kg live weight were evaluated throughout their lactation, kept in individual cages measuring 1.6×1.0 m². The feed given was in the form of commercial concentrate elephant grass silage, each 1400 g/day; the reference for the feeding amount was based on the results of preresearch conducted on the Sapera dairy goats. Feed was given twice a day, namely at 07.00 and 15.00 WIB. Provision of drinking water is made ad libitum by using a drinking bucket provided in each cage. Milking was done manually in the morning at 08.30 WIB.

Experimental design

Goats are kept during lactation (about six months). Individual milk production was recorded every day, while milk samples from each goat for observation were taken every week for two months of lactation. Milk samples were obtained immediately after taking the IRT sensing results. The parameters observed in this study included measurements of physicochemical characteristics, the number of somatic milk cells in each half udder, mastitis score using reagent from Bogor Agricultural University (IPB-1), and the result of IRT sensing of Sapera dairy goats on the udder.

Physicochemical characteristics of milk

Samples for somatic cell count were obtained from each nipple separately and cooled immediately, then transported in a box equipped with ice for immediate analysis. Samples were stored at -20 °C until further testing. Analysis of milk composition included fat content, solids non-fat (SNF), specific gravity, lactose, salts, protein, add water, freezing point, and acidity (pH) using MCC lactoscan (Milkotronic Ltd., Bulgaria).

Somatic cell count

Calculation of the number of somatic cells (SCC) using the Breed method was carried out by taking 0.01 ml of milk sample (using a Breed pipette), which was then distributed over a 1 cm^2 area (on a fat-free object glass). The preparations were awaited to dry, then fixed

over the flame. The milk fat was dissolved by immersing the object-glass in alcohol ether for two minutes and shaking it. The preparations were then stained with methylene blue Löffler for 1-2 minutes. The trials were then rinsed with water and immersed in 96% alcohol to remove the residual dye. After drying, the number of SCC ml^{-1} cells could be counted with a microscope with 1000 x magnification and observed for 30 fields of view. The formula calculates SSC:

$$
\frac{SCC}{ml} = mycrosscopy factor x a
$$

Where microscope factor is $10,000/\mu$ ² equal to 393,174 (microscopy model type MC300); a is means of somatic cells from 30 fields of view; and r is radius area of view (mm).

Mastitis test score

Mastitis test was carried out using an IPB-1 reagent (Faculty of Veterinary Medicine, IPB University). 2 ml of milk sample was mixed with 2 ml of IPB-1 reagent and homogenised for 15-30 seconds. The interpretation of the results obtained was grouped into five scores; namely, N: average or no reaction, T: trace or slightly slimy, tended to disappear with stirring, +1: dirty or thickened but did not form a gel, +2: gelled, moved as mass during mixing, +3: the gel forms a convex surface and adheres to the bottom of the holding cup. Scores of $+2$ or $+3$ are indicative of mastitis, according to the directions of Shearer and Harris (2003).

Infrared Thermography

IRT sensing results using an infrared camera brand FLIR A320 (FLIR Systems Co. Ltd., St Leonards, NSW, Australia) with an emissivity coefficient equal to 0.98. The results of the IRT sensing of each animal were taken on the udder (right and left udder). Thermal images of the udder were taken at a distance of 1 m from the skin of the udder. Udder temperature was analyzed by ThermaCAM Researcher Professional 2.10. ThermaCAM Researcher Professional is a robust real-time digital storage, measurement, and analysis software. Extremely versatile, researcher digitally stores and retrieves static and real-time infrared images, live IR digital video sequences, dynamic high-speed events and data directly from the FLIR IR camera allowing indepth and precise analysis of thermal events.

Statistical analysis

Collected physicochemical properties, milk somatic cell count, mastitis test, and IRT sensing results were analysed using the MIXED and CORR procedure from SAS (V. 9.1; SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Average milk quality, number of somatic cells, and results of IRT sensing on the right and left udders of the Sapera goat for each mastitis test score are presented in Table 1. In contrast, the description of the results of IRT sensing on the goat udder is illustrated in Figure 1. Several analysed parameters showed no difference in specific gravity between mastitis test scores (P>0.05). In contrast, other parameters, namely fat, SNF, lactose, protein, freezing point, pH, SCC, and IRT, were significantly different (P<0.05), especially between normal condition mastitis test scores with $+3$. This is due to damage to udder tissue infected, mastitis-causing an increase in SCC and changes in milk composition. Vural et al (2016) reported that infection due to mastitis would be transmitted from blood to milk so that there is an increase in the amount of protein, enzymes, and some milk minerals that cause changes in milk composition. Furthermore, efforts to balance the osmotic effect of milk due to infection require the role of mineral and lactose levels, which result in a decrease in mineral and lactose levels (Abdelgawad et al 2016). Several previous studies have also reported a reduction in milk lactose (Sharma et al 2014) and freezing point (Akdag et al 2017), which can be used as indicators of subclinical mastitis Further, Caboni et al (2017) reported that milk with high SCC had higher fat and protein content with a lower freezing point when compared to milk with low SCC.

Milk fat content of uninfected goats $(4.41 \pm 1.28\%)$ decreased compared to goats infected with mastitis category $+3$ (2.04 \pm 0.84%). Similar results were also reported by Kifaro et al (2009) on dairy goats. This is because the milk infected with mastitis has a very high increase in lipase enzyme activity resulting in the breakdown of milk fat and the release of free fatty acids that cause an unpleasant taste in milk (Uallah et al 2005). Likewise, the protein level of uninfected goats' milk (4.10±0.15%) decreased compared to goats infected with mastitis in the $+3$ category (3.89 \pm 0.14%). The same results were also reported by Khan and Khan (2006) in dairy goats. In milk infected with mastitis, there will be an increase in the proteolytic enzyme activity (plasmin), which causes extensive damage to milk proteins in the udder.

SNF levels also showed that goat's milk with a mastitis test score of $+3$ (8.24 \pm 0.29%) was lower than the normal mastitis test score $(8.65\pm0.32\%)$, a decrease in milk SNF in infected goat udders. Dependent on damage to udder tissue by invading pathogens leading to the reduction in the synthetic activity of the mammary glands (Ben Chedly et al 2010). Similarly,

Parameter	Normal		Trace		$+1$		$+2$		$+3$	
	Udder									
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Fat $(\%)$	4.41 ± 1.28 ^a	4.26 ± 0.80^{ab}	3.34 ± 1.03^{ab}	4.32 ± 1.58^{ab}	3.80 ± 1.72 ^{ab}	3.29 ± 0.72 ^{ab}	3.06 ± 1.05^{bc}	3.19 ± 0.82 ^{abc}	3.28 ± 0.48 ^{ab}	2.04 ± 0.84 ^c
SNF $(\%)$	8.65 ± 0.32^{ab}	8.88 ± 0.20^a	8.77 ± 0.29^a	8.48 ± 0.34 ^{abcd}	8.58 ± 0.28 ^{abc}	8.56 ± 0.44 ^{abc}	8.67 ± 0.48 ^{ab}	8.09 ± 1.05 ^d	8.17 ± 0.23 ^{cd}	8.24 ± 0.29 bcd
Specific gravity (g/ml)	1.03 ± 0.000^a	1.03 ± 0.000^a	$1.03 \pm 0.003^{\text{a}}$	1.03 ± 0.000^a	1.03 ± 0.002^a	1.03 ± 0.002^a	1.03 ± 0.004^a	1.03 ± 0.004^a	$1.03 \pm 0.002^{\text{a}}$	1.03 ± 0.000^a
Lactose $(\%)$	3.97 ± 0.14^{ab}	4.00 ± 0.09^a	3.94 ± 0.13^{ab}	3.82 ± 0.15^{bcd}	3.86 ± 0.12^{abc}	3.85 ± 0.20 ^{abc}	3.82 ± 0.13^{bcd}	3.81 ± 0.15^{bcd}	3.67 ± 0.10 ^d	3.70 ± 0.13 ^{cd}
Salts $(\%)$	0.64 ± 0.02 ^{ab}	0.66 ± 0.01^a	0.65 ± 0.02^a	0.62 ± 0.03 ^{abc}	0.63 ± 0.02 ^{abc}	$0.62 \pm 0.03^{\rm abc}$	0.63 ± 0.04 ^{abc}	0.60 ± 0.07 ^c	0.60 ± 0.02 ^c	0.60 ± 0.02 ^{bc}
Protein $(\%)$	4.10 ± 0.15^{ab}	4.22 ± 0.10^a	4.16 ± 0.14 ^a	4.02 ± 0.17 ^{abcd}	4.07 ± 0.13 ^{abc}	4.06 ± 0.22 ^{abc}	4.10 ± 0.23^{ab}	3.84 ± 0.50 ^d	3.86 ± 0.11 ^{cd}	3.89 ± 0.14^{bcd}
Add water $(\%)$	7.65 ± 4.01 ^{bc}	6.11 ± 1.71 ^c	6.49 ± 2.96 ^c	9.65 ± 3.72 ^{abc}	8.37 ± 2.94 ^{bc}	7.52 ± 4.83 ^{bc}	8.41 ± 4.18 ^{bc}	13.84 ± 12.37 ^a	12.29 ± 2.58 ^{ab}	11.66 ± 3.24^{ab}
Freezing point (°C)	$0.48 \pm 0.02^{\text{bcd}}$	-0.48 ± 0.01 ^d	-0.48 ± 0.01 ^d	$0.46{\pm}0.01^{\text{abcd}}$	$0.47 \pm 0.01^{\rm bcd}$	$0.48{\pm}0.02^{\text{bcd}}$	-0.48 ± 0.02 ^{cd}	-0.44 ± 0.06^a	-0.45 ± 0.01^{ab}	$0.45 \pm 0.01^{\rm abc}$
pH	6.92 ± 0.04^{ab}	6.92 ± 0.06^a	6.90 ± 0.04 ^{abc}	$6.90 \pm 0.09^{\rm abc}$	$6.86 \pm 0.13^{\rm abcd}$	6.80 ± 0.07^{bcde}	6.78 ± 0.08 ^{de}	6.80 ± 0.08 ^{cde}	6.75 ± 0.03 ^{de}	6.74 ± 0.03^e
SCC (x103)	321 ± 72^b	259 ± 70^b	356 ± 70^b	278 ± 73^{b}	477 ± 121 ^b	422 ± 119^b	749 ± 119^b	604 ± 131 ^b	$2690 \pm 1384^{\text{a}}$	$2120 \pm 938^{\text{a}}$
IRT $(^{\circ}C)$	37.24 ± 0.18^b	37.32 ± 0.28^b	37.27 ± 0.57^b	37.44 ± 0.30^b	37.42 ± 0.37^b	37.37 ± 0.39^b	38.12 ± 0.83 ^a	38.43 ± 0.34 ^a	38.34 ± 0.28 ^a	38.62 ± 0.34 ^a

Table 1. Average quality milk of Sapera dairy goat for each mastitis test score

Different superscripts on the same line for each mastitis test score showed significant differences (P<0.05). SNF= Solid non-fat, SCC= somatic cell count, IRT= infrared thermography

*P<0.05, **P<0.01, ns= no significant. SNF= Solid non fat, SCC= somatic cell count, IRT= infrared thermography

Figure 1. The description of IRT sensing results on goat udders with a normal milk mastitis test score (A) and a mastitis test score of +3 (B)

lactose levels in goat's milk with a normal milk mastitis test score $(3.97\pm0.14\%)$ decreased if the goat was infected with mastitis with a mastitis test score of $+3$ $(3.70 \pm 0.13\%)$. This is possible because lactose is synthesised in udder gland cells from glucose and galactose. Still, during inflammation, the secretory activity of cells is reduced due to damage to epithelial cells by leukocytes (Coulona et al 2002). The degree of acidity (pH) was lower in infected goat's milk with a mastitis test score of $+3$ (6.74 \pm 0.03%) than in goat's milk under normal conditions $(6.92 \pm 0.04\%)$, where these changes are associated with increased permeability of epithelial cells in the udder resulting in the transfer of components such as citrate and bicarbonate from blood to milk, causing an increase in pH. However, Chen et al (2010) reported no significant difference in the pH of goat's milk between normal and mastitis-infected milk, although higher levels of SCC in milk, if not induced by an apparent intramammary infection, would not cause changes in milk pH.

Results showed that the mastitis test scores using an IPB-1 reagent increased inline with the SCC scores and IRT sensing on the udder. A normal milk mastitis test score results of IRT sensing on goat udders is 37.24 \pm 0.18 °C and the SCC value is 321 \pm 72 x10³ cells mL^{-1}) increased if the goat are suggestive of intramammary infection (mastitis test score of +3) with IRT sensing on goat udders is 38.62 ± 0.34 °C and the SCC value is $2,120 \pm 938 \times 10^3$ cells mL⁻¹. An increase in SCC is a significant milk primary marker for detecting and diagnosing mastitis (Viguier et al 2009). Further Rainard et al (2018), SCC is a highly sensitive biomarker of mammary gland inflammation in which variations in SCC depend on leukocyte recruitment from blood to milk in response to inflammatory reactions elicited in udder tissue bacterial intrusion into the mammary gland. Under normal conditions, the amount of SCC in goat's milk varied between 210-1,120 $x10³$ cells mL⁻¹ (Leitner et al 2004). This happened because the mastitis test score was $+3$; the condition of the goat was in the subclinical mastitis phase and had

just experienced an acute inflammatory process. The inflammatory process due to mastitis begins through dilation of blood vessels, increasing blood flow to the infected udder, and increasing udder temperature. However, oedema reduces blood flow when it enters the chronic stage, lowering the temperature (Jones & Plassmann, 2002). Furthermore, studies involving endotoxin infusion into the mammary gland resulted in an increase in udder temperature of 1.5–2.0 °C within 24 hours after infusion (Hovinen et al 2008).

Milk production of individual Sapera goats during lactation is shown in Figure 2. As many as eight Sapera goats were used, it turned out that four of them showed a normal mastitis test score of +2 and four more with a mastitis test score of $+3$. The graph shows that the milk production of goats with a mastitis test score of $+3$ is lower than that of goats with a normal mastitis test score of $+2$. The average milk production of goats with a normal mastitis test score up to $+2$ for six months of lactation was $1,281\pm253$ ml/day, while for goats with a mastitis test score of $+3$, it was 957 ± 250 ml/day. According to Le Roux et al (2003), mastitis infection causes damage to the secretory epithelium of the mammary gland and affects the components of milk and milk production. The decrease in milk production due to mastitis in this study was also strengthened by the increase in the SCC value of milk. Sharma et al (2011) reported that high SCC content in milk is a significant indicator of infection in the mammary glands, resulting in decreased milk production.

Data on milk components (fat, SNF, lactose, salt, and protein), IPB-1 mastitis score, SSC, and IRT results were analysed by Pearson correlation $(p<0.05$ and p<0.01) to show the strength of the correlation between the various components of milk, presented in Tables 2 and 3. A fairly high and very significant positive correlation $(P<0.01)$ was found in both the right udder and left udder between levels of SNF-lactose (r=0.79- 0.89), SNF-salt (r=0.95- 0.97), SNF-protein (r=0.99), lactose-salt (r=0.81-0.87), lactose-protein (r=0.81-0.91), salt-protein (r=0.96-0.98), add water-freezing point

Figure 2. Graph of individual milk production (A-H) of Sapera goats during lactation. Goats A, B, C, and D obtained a normal mastitis test score of up to $+2$, while goats E, F, G, and H obtained a mastitis test score of $+3$

 $(r=1.00)$, IPB1-SCC $(r=0.70-0.74)$, IPB1-IRT $(r=0.70-0.74)$ 0.71), and SCC-IRT (r=0.62-0.65). As the main source of carbohydrates, Lactose controls milk volume by maintaining osmolarity through the rate of lactose synthesis in epithelial cells in the mammary glands (Zhao and Keating 2007). It can also affect the synthesis and secretion of other milk components (Shahbazkia et al 2010). Somatic cell count (SCC) is one of the most important indicators of milk quality. In contrast, SCC is an index of udder health that correlates with the level of infection in the mammary gland, whether subclinical, chronic, or acute mastitis (Kalaydzhiev et al 2021). The increase in milk SCC in response to udder infection causes a decrease in milk quality which is characterised by changes in the chemical composition of milk by decreasing casein, lactose, calcium, and increasing sodium, chloride, and serum protein (Pitkälä et al 2004).

Thermal condition of the surface of each organ reflects metabolic processes that are influenced by fluctuations in the amount and rate of blood supply, in which organs that experience hyperthermia and redness due to accelerated blood flow are used as early signs of inflammation (McCafferty et al 2011). The data at this time revealed that the results of IRT and SCC showed high scores in line with the increase in IPB1 scores. Udder with a mastitis test score of +1 or more had higher udder temperature and SCC than cattle in healthy conditions (Redetzky et al 2005). Therefore, this can explain the positive correlation obtained in this study between IRT, SCC, and IPB1. These results are similar to those previously reported in ruminants (Samara et al 2014). This is substantial evidence that combining IRT results with SCC and IPB1 parameters can be helpful in screening for subclinical mastitis in dairy goats.

A relatively high and very significant negative correlation was found between SNF-add water, SNFfreezing point, lactose-add water, lactose-freezing point,

salt-add water, salt-freezing point, protein-add water, and protein-freezing point. These results are similar to those reported in cattle (Dehinenet and Mekonnen 2013). According to Kurwijila (2006), an increase in added water can decrease the specific gravity and increase the freezing point of milk. Still, it affects the physicochemical quality of milk.

CONCLUSION

IRT sensing results showed that the temperature of goats with subclinical mastitis was 38.34-38.62 °C. Average milk production during lactation in goats with a mastitis test score of $+3$ (957 \pm 250 ml/day) was lower than in goats with a normal mastitis test score of $+2$ $(1,281\pm 253 \text{ ml/day})$. The correlation between the IRT right/left udder results with the mastitis test score, and the number of somatic cells of 0.62-0.71 indicates that the IRT sensing results can be a diagnostic method in screening for subclinical mastitis in dairy goats.

ACKNOWLEDGEMENT

All authors are thankful to Ir. Anneke Anggraeni M.Si., PhD, Mr Aseppriyadi, and all technical staff of the Goat Research Unit of Indonesian Research Institute for Animal Production Bogor Indonesia for their technical assistance. All authors are thankful to Dr. drh. Herwin Pisestyani, M.Sc. and all technical staff of the Veterinary Health Laboratory, Veterinary Health Division, Faculty of Veterinary Medicine IPB University, Mohammad Farhan Fauzan and Narkolas Indra Cahya as undergraduate students of the Faculty of Veterinary Medicine IPB University for the technical assistance provided in observing milk samples. The first author is grateful for the research scholar's financial support from the Indonesian Agency for Agricultural Research and Development.

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