UNIVERSITI PUTRA MALAYSIA

MILK COMPOSITIONS OF DAIRY COWS WITH CLINICAL AND SUBCLINICAL INTRAMAMMARY INFECTIONS

IDA AMALINA BINTI MAHADI

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FACULTY OF VETERINARY MEDICINE

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MILK COMPOSITIONS OF DAIRY COWS WITH CLINICAL AND SUBCLINICAL INTRAMAMMARY INFECTIONS

IDA AMALINA BINTI MAHADI

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Milk Compositions of Dairy Cows with Clinical and Subclinical Intramammary Infections", by Ida Amalina Binti Mahadi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project.

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DR. ROZAIHAN MANSOR

DVM (UPM), Ph.D (Glasgow)

Senior Lecturer,

Faculty of Veterinary Medicine

Universiti Putra Malaysia,

Serdang, Selangor

(Supervisor)

____

PROF. DR. ABDUL AZIZ SAHAREE

B.V.Sc. & A.H (Bombay), B.V.Sc. (Melbourne), M.Sc. (Edinburgh), Ph.D (UPM)

Professor,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

DEDICATIONS

In the name of Allah, The Most Benevolent, The Most Merciful

Mostly dedicated to:

Ayah, Ibu, Akak, Er, Aiman and Amirul.

Love you guys so much.

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LIST OF ABBREVIATIONS

UPM	=	Universiti Putra Malaysia
FPV	=	Faculty of Veterinary Medicine
UVH	=	University Veterinary Hospital
TPU	=	Taman Pertanian Universiti
IMI	=	Intramammary Infections
CMT	=	California Mastitis Test
SCC	=	Somatic Cell Counts
SNF	=	Soli non-fat
FFA	=	Free fatty acids
%	=	Percentage
et al.	=	et al. (abbr. Latin) et alii (and others)

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ABSTRAK

Abstrak daripada projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KOMPOSISI-KOMPOSISI SUSU LEMBU TENUSU DENGAN JANGKITAN INTRAMAMMARI KLINIKAL DAN SUBKLINIKAL

Oleh

Ida Amalina Binti Mahadi

2016

Penyelia: Dr. Rozaihan Mansor

Penyelia Bersama: Prof. Dr. Abdul Aziz Saharee

Pengeluaran dan kualiti susu boleh merosot dengan jangkitan intramammari (IMI) yang menyebabkan perubahan komposisi-komposisi susu. Kajian ini dijalankan untuk menentukan perubahan dalam parameter komposisi-komposisi susu dan untuk mengaitkan hubungan antara bilangan sel somatik (SCC) dan parameter komposisi-komposisi susu lembu tenusu dengan jangkitan intramammari dan tanpa jangkitan intramammari. Sebanyak 20 ekor lembu tenusu dari Taman Pertanian Universiti (TPU) dan Ladang Angkat (*Foster Farms*) dimasukkan di dalam kajian ini. Ujian California Mastitis (CMT) telah digunakan untuk mengenal pasti jangkitan inramammari subklinikal dalam lembu tenusu (n=10) manakala lembu tenusu yang sihat (n=10) telah digunakan sebagai kawalan negatif berdasarkan keputusan CMT (negatif, surih) tanpa tanda-tanda jangkitan intramammari klinikal. Malangnya, tidak ada kes jangkitan intramammari klinikal ditemui dalam kajian ini. SCC dan parameter komposisi-komposisi susu (susu lemak, protein, kasein, laktosa, jumlah pepejal, pepejal bukan lemak (SNF), asid lemak bebas (FFA), dan keasidan) telah ditentukan dan kasein, laktosa, SNF dan SCC ditemui akan berbeza dengan ketara antara kawalan dan kumpulan jangkitan intramammari subklinikal. Kasein, laktosa dan SNF juga didapati negatif dikaitkan dengan SCC. Kesimpulannya, perubahan ketara dalam parameter komposisi-komposisi susu boleh ditemui di dalam susu lembu tenusu dengan jangkitan intrammari subklinikal yang seterusnya menjejaskan kualiti susu.

Kata kunci: Jangkitan Intramammari (IMI), parameter komposisi susu, bilangan sel somatik (SCC), Ujian California Mastitis (CMT), kualiti susu.

ABSTRACT

An abstract from the project paper presented to the Faculty of Veterinary Medicine in the partial fulfilment of course VPD 4999 – Project

MILK COMPOSITIONS OF DAIRY COWS WITH CLINICAL AND SUBCLINICAL INTRAMAMMARY INFECTIONS

By

Ida Amalina Binti Mahadi

2016

Supervisor: Dr. Rozaihan Mansor

Co-Supervisor: Prof. Dr. Abdul Aziz Saharee

The production and quality of milk can deteriorate by intramammary infections resulting in milk compositions alteration. This study was done to determine the changes in the milk composition parameters and to correlate the relationship between somatic cell counts (SCC) and milk composition parameters of dairy cows with and without IMI. A total of 20 dairy cows from Taman Pertanian Universiti (TPU) and *Ladang Angkat* (Foster Farms) were included in this study. The California Mastitis Test (CMT) was used to identify subclinical IMI in dairy cows (n=10) while healthy dairy cows (n=10) were used as the negative control based on CMT results (negative, trace) with no clinical signs of IMI. However, no clinical IMI case was found in this study. The SCC and the milk compositions parameters (milk fat, protein, casein, lactose, total solid, solid non-fat (SNF), free fatty acids (FFA), and acidity) were determined and casein, lactose, SNF and SCC were found to be significantly different between control and subclinical IMI groups. Furthermore, casein, lactose and SNF were also found negatively correlated with the SCC. In conclusion, significant changes in milk compositions parameter can be found in milk of dairy cows with subclinical IMI which consequently affect milk quality.

Keywords: Intramammary infections (IMI), milk composition parameter, somatic cell counts (SCC), California Mastitis Test (CMT), milk quality.

1.0 INTRODUCTION

1.1 Background of the study

Milk which is also known as the secretion produced by animal's mammary gland especially cows, buffaloes, goats and sheep used for human consumption in a variety range of dairy products worldwide (Walstra *et al.*, 2006). Malaysia relies heavily on importation to satisfy domestic demand for dairy products. The dairy product import value had increased from RM69 million in 1970 to RM1.2 billion in 2014. Although the milk production has increased over the past decades, Malaysia is still unable to meet the demand (Rachel & Chubashini, 2015). Between 1990 & 2005, consumption of fresh whole milk increased by 33% from 32.9kg/capita to 43.5kg/capita (Boniface & Umberger, 2012). The increasing awareness on nutritional benefits coupled with increasing preference on dairy products has contributed to increase demand of dairy product in Malaysia. Therefore, the rising demand has driven the government to formulate policies and suggest steps to meet the need (Rachel & Chubashini, 2015).

The production and quality of milk can be influenced by various factors such as disease occurrence namely intramammary infections (IMI) or mastitis (Payne and Wilson, 1999). The IMI will not only cause an adverse effects on the quality of milk by decreasing the synthetic capacity of mammary gland such as decreased synthesis of casein and lactose (summarized by Urech *et al,*. 1999; Auldist & Hubble, 1998; Pyörälä, 2003; and Akers, 2002) but it also caused the damaged mammary tissue to be increased in vascular permeability. As a result, milk composition is altered due to leakage of blood constituents, serum protein enzymes, and salts into the milk (Østerås, 2000; Harmon, 1994). There are several challenges faced by the Malaysia's dairy sector such as lack of skills and training, low breed performance and inadaptability, poor dairy farm management and inadequate nutritious feed, high input and feed costs (Rachel & Chubashini, 2015).

IMI is the most prevalent disease in dairy cattle which raised an economically concerned that is responsible for major losses in dairy industry especially subclinical form IMI (Tomazi *et al.*, 2015). Subclinical form IMI does not cause visible changes in the milk or the udder but it decreases the production and alters the milk compositions with the presence of bacteria in the secretion. It also results in an influx of inflammatory cells (somatic cell) which often detected based on increase of SCC in the milk (Ruegg and Erskine, 2014).

Clinical form of IMI is often characterized by abnormal milk secretion (presence of clots, flakes or blood) which may be accompanied with or without abnormalities in the mammary gland (hard, pain or swelling of the cow's udder) or systemic signs such as lethargy or anorexia and increased in rectal temperature and these would aid in the diagnosis of IMI (Harmon, 1994; Sandholm *et al.*, 1995; Pinzón-Sánchez & Ruegg, 2011; and Ruegg & Erskine, 2014).

The IMI causative agents can be categorized into minor or major pathogens (Eberhart *et al.*, 1987; Harmon *et al.*, 1986; Hogan *et al.*, 1987; and Schalm *et al.*, 1971). Major pathogens such as *Staphylococcus aureus*, *Streptococci (agalactiae, dysgalactiae, uberis)*, *Escherichia coli* and *Klebsiella* spp are the most common causative agent which causes clinical form IMI (Djabri *et al.*, 2002; Nielsen, 2009) and give extensive changes to the milk compositions (Harmon, 1994). Coagulase-negative staphylococci and *Corynebacterium bovis* are considered as minor pathogens causing only moderate infection and most often associated with subclinical form IMI (Djabri *et al*., 2002; Harmon, 1994).

1.2 Overall Objectives of the study

1. To determine the changes in the milk compositions parameter of dairy cows with clinical and subclinical intramammary infections (IMI).
2. To correlate the relationship between somatic cell counts (SCC) and milk compositions parameter in the milk of dairy cows.

1.3 Justifications of the study

1. The clinical and subclinical intramammary infections may alter the milk composition by changing the quality and hygienic value of milk.
2. The clinical and subclinical intramammary infections should be identified at an early stage as to maintain the milk quality and productivity of dairy industry.
3. There is a high demand of milk yield and milk quality in dairy cows, thus preventative measures is essential to avoid negative consequences of clinical and subclinical intramammary infections that may lead to economic losses to producers and health problems to consumers due to antimicrobial residue present in the milk.

1.4 Hypotheses of the study

Ho: There is no significant difference in the milk composition parameters between healthy dairy cows and infected dairy cows with clinical and subclinical IMI.

Ha: There is significant difference in the milk composition parameters between healthy dairy cows and infected dairy cows with clinical and subclinical IMI.

Ho: There is no correlation between SCC and the milk composition parameters in the milk of dairy cows with and without IMI.

Ha: There is correlation between SCC and the milk composition parameters in the milk of dairy cows with and without IMI.

2.0 LITERATURE REVIEW

2.1 Intramammary Infections (IMI) in Dairy Cows

Intramammary infections (IMI) or mastitis is the inflammation of the mammary gland and the most prevalent disease in dairy cows which is responsible for major dairy industry losses, especially in the subclinical form of mastitis (Tomazi *et al.,* 2015). It occurs when pathogens enter the teat canal and cause damage to the mammary epithelial cells which lower the synthesis of milk components. As a result, the dead and sloughed off epithelial cells and leukocytes (PMN) will be present in milk. This condition caused higher SCC present in milk (Viguier *et al*., 2009).

IMI is characterized by physical, chemical and bacteriological changes in the milk as well as pathological changes in the glandular tissue of the udder (Sharma, 2007). Clinical mastitis is characterized by abnormal changes of milk and mammary gland (heat, pain, swelling) while subclinical mastitis which is characterized by the absence of physical signs (heat, swelling, discoloration of the udder, abnormal secretion and systemic reactions), but results in increased of somatic cells counts in milk secreted from affected udders due to influx of inflammatory cells (somatic cells) (Fuenzalida *et al.,* 2015 and Peters *et al.,* 2015). Both clinical and subclinical mastitis are induced after pathogenic microorganisms gain an entrance into the udder through the teat canal, overcome the cow's defence mechanisms and begin to multiply and producing toxins that are harmful to the mammary gland. Damaged mammary tissue causes increase in vascular permeability. As a result, milk composition is altered due to leakage of blood constituents, serum proteins, enzymes, and salts into the milk.

2.2 California Mastitis Test (CMT)

California Mastitis Test (CMT) is an inexpensive, simple and rapid qualitative cow side test which can be performed during milking and give instant results for subclinical IMI screening.

The gel or slime formation is based on the CMT reagent that reacts with DNA of leukocytes (SCC) after disrupting the cellular membrane. The gel or slime formation will be thickened in relation to higher amount of the membrane being destroyed. Therefore, the number of somatic cell present can be roughly estimated.

According to Sargeant *et al.*, (2001) a recent study comparing CMT with SCC in newly calved dairy cows and the CMT was found to be a suitable marker for daily herd monitoring programme in detecting fresh cows with IMI (Pyorala, 2003). Instead of IMI inspection, CMT can also be applied to assess the efficacy of treatment to identify cows for treatment if dry cow therapy is used at dry off period. According to (Poutrel and Rainard, 1981), CMT was found to correctly identify 75-80% of the dairy cows that required therapy depending on the study and type of the IMI pathogen.

2.3 Somatic Cell Count (SCC)

Somatic cell count (SCC) in milk has been used widely as an indicator for cow's udder health and the quality of milk especially for determining the extent of the occurrence of subclinical IMI in dairy cows since the development of rapid electronic cell counting techniques. It represents an approximate number of leukocytes or white blood cells in milk that include macrophages, lymphocytes and polymorphonuclear neutrophils (PMN) (Kretschmer, 2007 and Harmon, 2001). Burvenich *et al,* 2000 stated that the most predominant cell type in normal milk were macrophages which made up 30% and 74% of the total cells from healthy glands. At early inflammatory stage, over 90% of the polymorhonuclear cells will migrate into milk to engulf and kill the bacteria (Harmon and Heald, 1982; Nickerson and Pankey, 1984; Burvenich *et al.*, 2000; and Paape *et al.*, 1979). Harmon (2001) added, the PMN also will engulf other particles such as fat globules and casein during inflammation. There were studies identifying cell types in milk made by (Lee *et al*. 1980), which showed that epithelial cells were infrequently found with range of 0 to 7% of the cell population in udder secretions and those come from the dry gland. Thus, increased in SCC at the end of lactation is not due to sloughing epithelial cells.

Inflammation is initiated upon entry of bacteria into the mammary gland which is associated with the influx of white cells from the bloodstream, altered secretory function and changes in the volume and composition of milk secretion. Bacteria or inflammatory mediators will produce toxin and damage tissue thus resulting in lower synthesis of milk (Harmon, 1994). According to Hurley (2010), SCC secretes proteolytic enzyme (plasmin) which is responsible for degradation of casein. It would also generate oxygen radicals which hydrolyse lipids and change the taste and smell of the milk. In addition to elevated SCC, infection causes increased permeability of blood vessels and mammary epithelium which leads to the leakage of blood into the milk and the movement of some normal milk components out from the alveolar lumen into the perivascular space (Harmon, 1994).

2.4 Bovine Milk Compositions

The cow's mammary gland is made up of a remarkably sensitive tissue that has the capability to produce large volume of secretion which is known as milk under normal or healthy conditions. Dairy cow's milk contains organic or inorganic components such as fat, protein, casein, lactose, total solid, solid non-fat, urea, citric acid and free fatty acid which makes the milk composition economically important to milk producers and processors especially in cheese production. Previous study has stated that in bulk milk, the major nutrients content were fat, protein and lactose at approximately 4.3%, 3.4% and 4.8%, respectively (Swedish Dairy Association, 2009; Walstra *et al*., 2006; Wickström, 2009). The highest component in bovine milk is total solids at 13.7% while the lowest component is free fatty acids (FFA) at 0.22-0.33% (Table 1).

The changes in composition of milk are one of the consequences of IMI in dairy cows (Gianneechini *et al*., 2002). It leads to a reduction of lactose and butter fat yield. However, the milk protein levels will increase slightly with IMI but of lower quality, with increased levels of globulin and decreased casein (Andrews *et al*., 2008; Blowey and Laven, 2004). According to (O'Rourke and Blowey, 2004), the inflammatory changes in the mammary gland influenced the process of milk synthesis both quantitatively and qualitatively. The changes in constituents of milk affect the major components such as lactose, fat, proteins as well as fatty acids, protein fractions, caseins, whey proteins, anions and cations, conductivity enzymes, and others.

The IMI initiates an inflammatory reaction which lead to decreased synthesis of milk components and an increased leakage at the tight junctions of the blood-milk barrier. Therefore, this implies that less lactose and caseins are being synthesized but the levels of whey proteins and salts are increased in the milk (Wickström, 2009;

Pyörälä, 2003; and Sandholm *et al.*, 1995). IMI alters the compositions of milk and the extent of this various compositional alteration depends on the inflammatory response (Kitchen, 1981; Korhonen *et al.*, 1995, Pyorala 2003). The degree to which it changes the composition of milk based on the pathogenicity of bacteria causing IMI and the degree of tissue damage in the mammary gland especially epithelial area.

Therefore, due to the strong relationship that exist between some of the inflammatory or compositional changes in milk with the presence of infection, the measurement of certain milk components is being used to monitor milk quality and cow's udder health (Craven & Williams, 1985; Mattila *et al.*, 1985 and Kitchen, 1981).

Table 1: Average percentage of bovine milk composition parameters (Manoj, 2012)

Milk compositions	**Fat**	**Protein**	**Total solids**	**Solid non-fat (SNF)**	**Casein**	**Lactose**	**Free fatty acids (FFA)**
Average %	3.6-4.9	3.3-3.4	13.7	8.8	2.6	4.9	0.22-0.33

3.0 MATERIALS AND METHODS

3.1 Selection of farm and animals

A total of 30 lactating cow ranging from 4-12 years old at 80-200 days of lactation, Friesian crossed Jersey and Friesian crossed Sahiwal from TPU, UPM and different Ladang Angkat, FPV, UPM were included in this study. The animals with subclinical IMI were selected based on the CMT results while the animals with clinical IMI were selected based on observation and palpation of the cow's udder. The dairy cows with positive CMT results of score more than 1+ (2+and 3+) (5 cases from each score without having any mammary gland abnormalities) with normal milk appearance were chosen as subclinical IMI group (n=10), while the dairy cows with observable inflammation on the infected quarter such as heat, pain, redness or swelling and presence of clots in the milk samples (Tabrizi *et al*., 2008) were chosen as clinical IMI group (n=10). Those healthy dairy cows (which do not exhibit any clinical findings of clinical or subclinical IMI) and negative for CMT results of score less than 1+ were chosen as control group in this study.

3.2 California Mastitis Test (CMT)

Teat was aseptically cleaned with alcohol and the first stream of milk from each quarter was discarded. Approximately 2ml of milk from each quarter were collected into the clean CMT paddle with 4 cups labelled A, B, C, and D. An equal amount of CMT reagent was then added into the milk before the paddle was rotated not more than 10 seconds. Observation was made for any gel or slime formation and its viscosity. The CMT results were interpreted as; Negative = healthy mammary gland, 0 (traces) = normal mammary gland, 1+ (positive) = mildly infected with positive result, 2+ (gel) = infected mammary gland with distinct positive result, 3+ (clumps) = heavily infected mammary gland (Table 2).

Table 2: Interpretation and Scoring of the CMT (Managing Quality Milk, Institut de technologie agroalimentaire de La Pocatière, 1998)

CMT Score	Meaning	Description of Reaction	Somatic Cell Range	Interpretation
N	Negative	Mixture remains liquid, homogeneous, with no evidence of thickening.	0 – 200,000 cells/ml	Normal and healthy mammary gland
T	Trace	Slight thickening seen when paddle is tipped back and forth. Trace reactions tend to disappear with continued rotation of the paddle.	150,000 – 500,000 cells/ml	Normal and healthy mammary gland
1	Weak Positive	A distinct thickening of the liquid but no tendency toward gel formation. Thickening may disappear after prolong rotation (>20 seconds).	400,000 – 1,500,000 cells/ml	Mildly infected with positive result
2	Distinct Positive	Mixture thickens immediately, and gel formation is suggested. As the mixture is swirled, it tends to move in toward the centre, exposing the bottom of the outer edge of the cup, levelling out and covering bottom of well when motion stops.	800,000 – 5,000,000 cells/ml	Infected mammary gland with distinct positive result
3	Strong Positive	A gel is formed, which causes the surface of the mixture to become elevated like a partially fried egg.	> 5,000, 000 cells/ml	Heavily infected mammary gland

3.3 Milk Samples Collection

The cow's udder and teat were cleaned using water to remove dirt. Next, the milk was stripped the first few streams of milk were discarded. Approximately about 100ml of milk samples were collected aseptically from quarter of dairy cows with or without IMI (clinical IMI; presence of clots in the milk samples and subclinical IMI; positive CMT result, 2+ and 3+) and stored at 4°C for further analyses within 24 hours.

3.4 Laboratory Analysis

3.4.1 Somatic Cell Counts (SCC) Analysis

The SCC was determined by flow cytometry system using FOSS Fossomatic Minor™ machine (Foss Electric, Hillerød, Denmark).

3.4.2 Milk Compositions Analysis

The milk composition parameters (milk fat, protein, casein, lactose, total solid, solid non-fat, free fatty acids, and acidity) were analysed by infrared absorption system using FOSS Milkoscan FT2™ machine (Foss Electric, Hillerød, Denmark).

3.5 Data Analysis

Data analysis was done using IBM SPSS version 20. The mean of SCC and milk compositions parameter between group with and without IMI was compared using Independent sample T-test. The correlation between SCC and milk compositions parameter was determined using Pearson correlation and $P < 0.05$ was considered significant.

4.0 RESULTS

The udder appearances and CMT results of dairy cows in TPU and Ladang Angkat, UPM are tabulated as in (Table 3). Most of the IMI cases found in TPU were subclinical form (n=6) while in 4 cases of subclinical IMI were found in Ladang Angkat.

The mean values for SCC and milk compositions parameter are also recorded as in (Table 4). Based on the result, the means of milk composition parameters of fat, protein, total solid, urea, free fatty acids, and acidity were not significantly different ($p>0.05$) between the control and the group of dairy cows with subclinical IMI. However, there was significantly difference ($p<0.05$) for the mean of milk composition parameter of casein, lactose, and SNF with p value of 0.015, 0.007, and 0.003 respectively in between the two groups. Likewise, the mean for SCC showed significant difference between the group of dairy cows without IMI and the group dairy cows with subclinical IMI (Table 3).

However, no clinical IMI case was found in this study. The correlations between SCC and milk composition parameters are recorded as presented in (Table 5). Based on the result, the SCC was negatively correlated with casein, lactose and solid non-fat ($p<0.01$) with the correlation coefficient value of -0.576, -0.676, and -0.722 respectively.

Table 3: Udder appearance and CMT results of dairy cows at TPU and Ladang Angkat, UPM

No.	Animal ID	Udder appearance	CMT Results		IMI Diagnosis	Farm
			L	R		
1.	**T024**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	3+ 0	Subclinical IMI	TPU
2.	**T058**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	3+ 2+	0 0	Subclinical IMI	TPU
3.	**T118**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	2+ 2+	3+ 1+	Subclinical IMI	TPU
4.	**T133**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	2+ 0	0 0	Subclinical IMI	TPU
5.	**M053**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	2+ 0	1+ 0	Subclinical IMI	TPU
6.	**50410**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	0 3+	Subclinical IMI	TPU
7.	**00859**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	3+ 3+	Subclinical IMI	En. Razlan

8.	**560**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 2+	2+ 2+	Subclinical IMI	En. Razlan
9.	**00437**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	- 1+	3+ 2+	Subclinical IMI	En. Razlan
10.	**4851**	All the quarters appeared normal no inflammation observed but the right fore quarter slightly warm upon palpation. Milk consistency normal and no clots.	- 1+	1+ 3+	Subclinical IMI	En. Razlan
11.	**T083**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	1+ 0	Non IMI	TPU
12.	**T092**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	0 0	Non IMI	TPU
13.	**T099**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	1+ 0	Non IMI	TPU
14.	**T018**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	0 0	Non IMI	TPU
15.	**T105**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	0 0	Non IMI	TPU

16.	**T123**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 1+	0 0	Non IMI	TPU
17.	**Brown**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 0	0 1+	Non IMI	En. Baghwan
18.	**White**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	1+ 1+	1+ 0	Non IMI	En Baghwan
19.	**Black White 1**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	1+ 0	0 1+	Non IMI	En. Baghwan
20.	**Black White 2**	All the quarters appeared normal no inflammation observed. Milk consistency normal and no clots.	0 1+	1+ 1+	Non IMI	En. Baghwan

Table 4: Somatic cell counts and milk compositions parameter of dairy cows with and without IMI (Mean ± SE)

Milk compositions parameter	**Without IMI, Mean ± SE**	**With subclinical IMI, Mean ± SE**	**With Clinical IMI, Mean ± SE**	**P value***
SCC ($x10^3$/ml)	175.4000 ± 36.50151	3331.0000±200.85855	-	**0.000***
Fat (%)	4.2390 ± 0.69939	4.2540 ± 0.62912	-	0.987
Protein (%)	3.3850 ± 0.10507	3.3610 ± 0.07913	-	0.857
Casein (%)	2.5260 ± 0.11944	2.0820 ± 0.11515	-	**0.015***
Lactose (%)	4.4950 ± 0.05252	3.9840 ± 0.14766	-	**0.007***
Total solid (%)	12.7130 ± 0.60530	11.7250 ± 0.58677	-	0.256
SNF (%)	8.6770 ± 0.12958	7.5770 ± 0.26717	-	**0.003***
FFA (mmol/100g fat)	0.57870 ± 0.066207	0.44490 ± 0.064101	-	0.164
Acidity (°Therner)	17.1340 ± 1.29518	17.4340 ± 1.04950	-	0.859

*significant at p value < 0.05

Table 5: Correlation between somatic cell counts and milk compositions parameter of dairy cows with and without IMI

Milk compositions parameter	**SCC**	**Fat level**	**Protein level**	**Casein level**	**Lactose level**	**Total solid**	**SNF**	**FFA**	**Acidity of milk**
SCC		0.023	-0.042	**-0.576****	**-0.676****	-0.282	**-0.722****	-0.319	0.052
Fat level	0.023		-0.395	-0.394	-0.216	0.888**	-0.296	0.188	-0.019
Protein level	-0.042	-0.395		0.462*	-0.008	-0.228	0.333	0.143	0.010
Casein level	**-0.576****	-0.394	0.462*		0.616**	-0.069	0.863**	0.130	0.131
Lactose level	**-0.676****	-0.216	-0.008	0.616**		0.211	0.902**	0.281	-0.459*
Total solid	-0.282	0.888**	-0.228	-0.069	0.211		0.150	0.359	-0.210
SNF	**-0.722****	-0.296	0.333	0.863**	0.902**	0.150		0.302	-0.236
FFA	-0.319	0.188	0.143	0.130	0.281	0.359	0.302		-0.166
Acidity of milk	0.052	-0.019	0.010	0.131	-0.459*	-0.210	-0.236	-0.166	

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Table 6: Intramammary Infections * Farm Crosstabulation

			Farm		Total
			TPU	Ladang Angkat	
Intramammary Infections (IMI)	**Normal**	**Count**	6	4	10
		% within Intramammary Infections	60.0%	40.0%	100.0%
		% within Farm	50.0%	50.0%	50.0%
		% of Total	30.0%	20.0%	50.0%
	Subclinical	**Count**	6	4	10
		% within Intramammary Infections	60.0%	40.0%	100.0%
		% within Farm	50.0%	50.0%	50.0%
		% of Total	30.0%	20.0%	50.0%
Total		**Count**	12	8	20

% within Intramammary Infections	60.0%	40.0%	100.0%
% within Farm	100.0%	100.0%	100.0%
% of Total	60.0%	40.0%	100.0%

Table 7: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.000[a]	1	1.000		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.000	1	1.000		
Fisher's Exact Test				1.000	.675
Linear-by-Linear Association	.000	1	1.000		
N of Valid Cases	20				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 4.00.

b. Computed only for a 2x2 table

5.0 DISCUSSION

5.1 Milk composition parameters

The parameter of fat in the milk of dairy cows showed no significant difference ($p<0.05$) between the control and subclinical IMI groups and this is not in accordance with (Andrews *et al.*, 2008) that found a decrease in fat concentration of milk with IMI. A study done by Kitchen, (1981) showed decreased in fat yield in milk infected with IMI due to decline of milk production. Another study using 290 lactating local zebu and Holstein-Zebu crossbred cows demonstrated that there was a significant difference in fat composition between non IMI (control) and clinical IMI ($t=3.9644$, $p<0.05$) and between subclinical IMI and clinical IMI ($t=4.3891$, $p<0.05$) stating breed was not a confounding factor affecting fat composition (Alemu *et al.*, 2013).

Many factors such as type and quantity of feed, breed, stage of lactation, lactation numbers and seasonal changes affect the milk fat. This is supported by Hibbitt *et al.*, (2008) which stated that dairy cow's milk fat content varied widely between and within the breed and it was influenced by diet and environment factors. However, in this study, the factors such as lactation stages and lactation number were unable to be determined due to poor record keeping.

Most studies stated that an increased in protein content cause significant changes in milk composition parameter particularly during IMI. This was supported by the study made by Batavani, (2007), which showed the percentage of protein fractions was significantly different between normal and SCM milk ($p<0.01$). However, the result of this current study was not statistically significant ($p<0.05$) at a value of 0.857. Therefore, this result was not in agreement with the previous study indicating that there is a slight increase in protein content in IMI cow's milk (Hortet & Seegers, 1998a & 1998b; and Nielsen, 2009).

An increased in milk protein during IMI. It has been attributed by an influx of blood-borne proteins (serum albumin and immunoglobulins), the minor serum proteins, transferring a-macroglobulin into the milk coupled with a decrease in casein (Auldist *et al.*, 1995; Auldist & Hubble, 1998 and Holdaway, 1990). Rao, 1999 added that the increase of total protein in subclinical milk might be due to increased non-casein proteins (blood proteins) in the affected quarters.

Proteins in milk are primarily made up of casein which is approximately 80% and whey protein at approximately 20%. Caseins are mainly comprised of αs1-, αs2-, β- and κ-casein in an approximate ratio of 4:1:4:1 and they will precipitate after rennet addition or in an acidic solution (Davies & Law, 1980; Farrell *et al.*, 2004; Walstra & Van Vliet, 1986). Meanwhile, whey proteins are composed of β-lactoglobulin 50%, α-lactaalbumin 20%, immunoglobulin 11%, and bovine serum albumin 6%, as well as others 13% (Farrell *et al.*, 2004). There is significant difference observed for casein in this study ($p<0.05$) at a value of 0.015. It has been predicted that the casein will show significant changes in dairy cow's milk since most of the previous studies mentioned that there will be a reduction in casein during IMI. This was supported by a study made by (Wickström, 2009), which showed that the contents of casein was lower (2.54±0.11 g/100ml) in the high SCC group compared with low SCC group (2.63±0.16 g/100ml). Casein acts as one of the important coagulation properties in milk important for cheese production. Increase in SCC during IMI will increase the coagulation time which lowers milk quality for cheese production.

Lactose is a disaccharide and the major carbohydrate in milk (Wickström, 2009). It is made up of one molecule glucose and one molecule galactose. Its concentration is approximately at 4.8% in bovine milk, which is relatively higher compared with other mammals and is the most consistent among any constituent due to its large influence maintaining the osmolality of milk (Hibbitt *et al.*, 2004 and Andrews *et al.*, 2008). However, (Sjaastad *et al.*, 2003) stated that the lactose content is still 30% lower than human milk. In this study, there was a significant difference of lactose parameter ($p<0.05$) with the value of 0.007. This is in agreement with most studies which mentioned that there will be a decrease in the concentration of milk lactose due to IMI (Auldist *et al.*, 1995; Auldist and Hubble, 1998). This is because during mastitis, the tissue is damaged and synthetic ability of the enzyme systems is decreased, thus the biosynthesis of lactose also decreases (Pyorala, 2003).

Study on the milk composition of clinical, subclinical and normal milk involving 290 lactating local and crossbred dairy cows was demonstrated by (Alemu *et al.*,) 2013 who found significantly lower lactose content in clinical milk as compared to subclinical and normal milk of cross bred dairy cows (4.76125 vs. 4.91175 vs. 5.173077). Meanwhile, the lactose content of local breed dairy cows in subclinical and normal milk was slightly higher than the lactose content of crossbred dairy cows (4.981429 vs. 5.297). Furthermore, (Shitandi *et al.*, 2005) mentioned that parity had significant influence ($p<0.05$) on lactose content. We can conclude that the lactose content is varied among the breed and parity. Therefore, it is not a suitable indicator to indicate IMI in dairy cows.

Bovine milk comprises of 12.7% total solids that are composed of fat and solid non-fat while the remaining 87.3% is water. Malek dos Reis *et al.*, 2013 stated that

IMI caused by mastitis-causing pathogens reduced the total solids content of milk. It was supported by (Bhoyar, 2008) who mentioned that the decrease in total solids is due to decrease in fat % and SNF % in subclinical milk. There is a significant reduction of solid non-fat (SNF) in milk with subclinical IMI ($p<0.05$) at a value of 0.003. A study on compositional changes in milk constituents associated with elevated SCC by (Harmon, 1994, Eberhart *et al.*, 1987 and Kitchen, 1981) showed a decrease in SNF in milk with higher SCC (8.8%) as compared with SNF in normal milk (8.9%) considering the 99% of normal milk. The average of SNF decreases may due to decrease in lactose content in mastitis milk (Rao, 1999).

The free fatty acids (FFA) content in subclinical milk is significantly reduced to the mean value of 0.44490 ± 0.0641 but it is not statistically different. The result of the current study was inconsistent with (Harmon, 1994 and Kitchen, 1981) who stated that there will be increased FFA in milk with high SCC. Harding, 1995 mentioned the high levels of FFA produced by the action of lipase on milk fat, resulting milk off-flavors (rancid flavors).

The acidity of milk showed no significant difference between normal and subclinical group even though the acidity of subclinical milk was higher compared with normal milk, it was not statistically different. However, this result was in accordance with study made by Batavani 2007 who confirmed that the pH was significantly higher in the subclinical mastitis milk than in the normal ones ($p<0.01$). The result also was consistent with the results of previous reports made by (Kitchen, 1981; Sena and Sahmani, 2001; Wielgosz-Groth and Groth, 2003) stated that the pH of subclinical mastitis milk was higher than that of normal milk. According to Rao, 1999 the pH value increases is due to increased permeability of the gland to blood components resulting the high value and alkaline salts in milk.

There was correlation found between SCC and the milk composition parameters (Table 5). The SCC was found to be negatively correlated with casein, lactose and SNF. As a result, we can conclude that an increase in SCC will decrease casein, lactose and SNF with value of -0.576, -0.676, and -0.722 respectively. The current result reflected with most studies shown that there was a relationship between SCC and other milk composition parameters. Numerous studies have shown that high SCC was associated with changes in the milk composition in the quarter, cow composite and bulk milk (reviewed by Auldist & Hubble, 1998), such as decreased contents of lactose (Kitchen, 1981), caseins (Barbano *et al.*, 1991) and an increase of sodium (Rogers *et al.*, 1989). Alteration in chemical composition of milk and different degrees of increment or decrement with respect to types of mastitis from the milk collected from normal cows (non IMI cows) in the present study was in agreement with the different works.

5.2 SCC parameter

The SCC in subclinical milk was significantly increased in subclinical milk which reflected most studies. Harmon, 1994 added a higher elevation of SCC was abnormal and as an indication of inflammation in the udder. The mean value of SCC in normal milk was 175.4000±36.501 while the mean value of SCC in subclinical milk was 3331.0000±200.858 which was 19-fold higher than normal milk. According to (Leslie, 2003) the bulk tank counted below 200,000 indicated an excellent udder health, while over 500,000 indicated a definite problem with subclinical mastitis.

(Schukken *et al.*, 2003) added that the milk with an SCC of more than 400,000 was unfit for human consumption by the European Union.

The SCC can, however, be affected by factors other than mastitis, such as number of lactations, stage of lactation, season and milking frequency. Thus, it is not the ultimate marker of milk quality, which has resulted in studies searching for more sensitive and specific markers (Pyörälä, 2003).

6.0 CONCLUSION AND RECOMMENDATIONS

Overall from this study, we could observe that there were certain milk composition parameter like casein, lactose and solid non-fat that were affected by subclinical IMI. This is mostly in accordance with other previous studies. Thus, we were able to meet the first objective of this study. Nevertheless, the milk composition parameter also can be affected by other factors such as breed, age, type of feed and lactational stages. Additionally, from this study, the SCC was found to have a strong relationship with certain milk composition parameter. It was found that the milk composition parameter of casein, lactose and SNF were negatively correlated with SCC in milk of dairy cows with and without IMI. The casein, lactose and SNF will be decreased in high SCC.

We were also able to meet the second objective of this study. As a result, it can be concluded that casein, lactose and SNF parameter can be used as one of the suitable indicator to determine IMI not only for dairy cows but also for other milk-producing animals in future.

As a recommendation of future study, it is encouraged to include a larger sample size from different herds of dairy cows in certain areas for a better statistically significant results considering a few confounding factors like breed, age, type of feed and lactational stage. Further study on other indicator of IMI in dairy cows, for examples changes in the level of protein fractions in milk such as α-lactalbumin, β-lactoglobulin and albumin or changes in the level of enzyme, such as N-acetyl-β-D-glucosaminidase (NAGase), beta-glucuronidase and catalase can also be carried out to determine the most appropriate indicator of IMI in dairy cows, especially subclinical

IMI instead of using the current gold standard method, such as SCC and bacteria isolation and identification.

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APPENDICES

APPENDIX A

CMT – Determination of subclinical IMI

APPENDIX B

250ml Bottle – Milk sample container

APPENDIX C

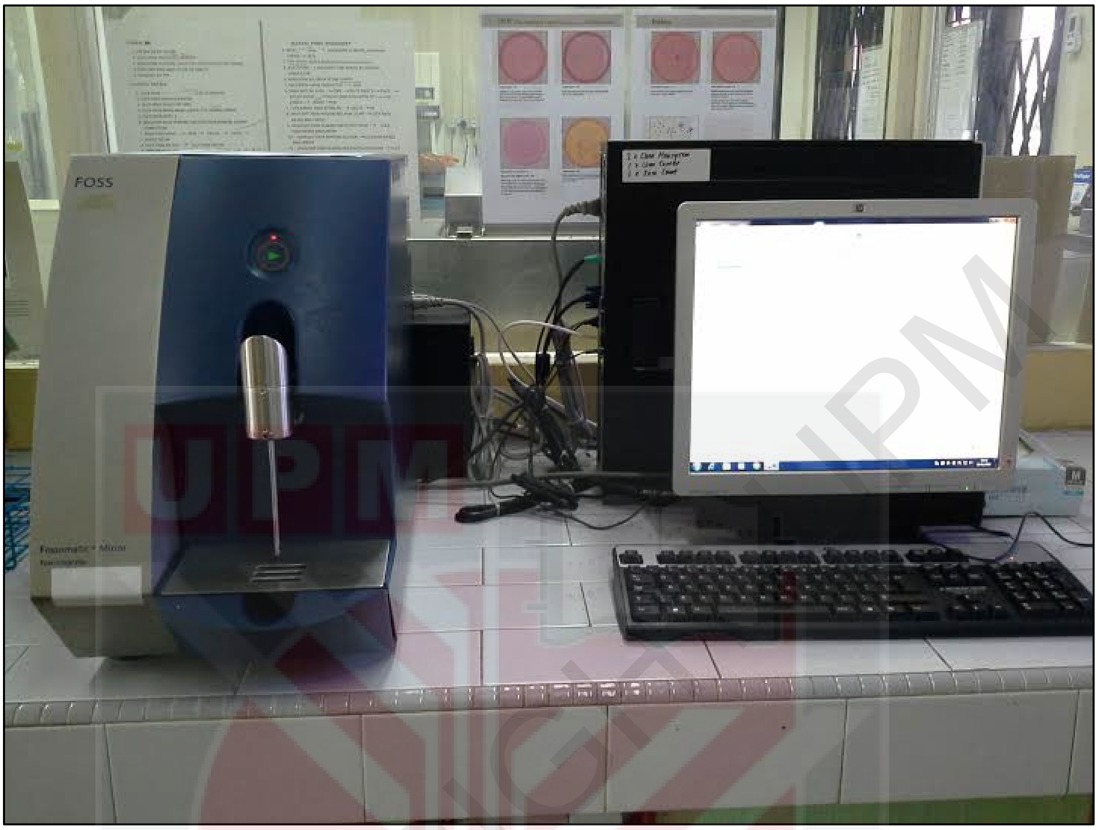
Chiller – Milk samples storage at 4°C

Chiller – Milk samples storage at 4°C

APPENDIX D

FOSS Fossomatic^{TM} Minor – Somatic Cell Count Analysis

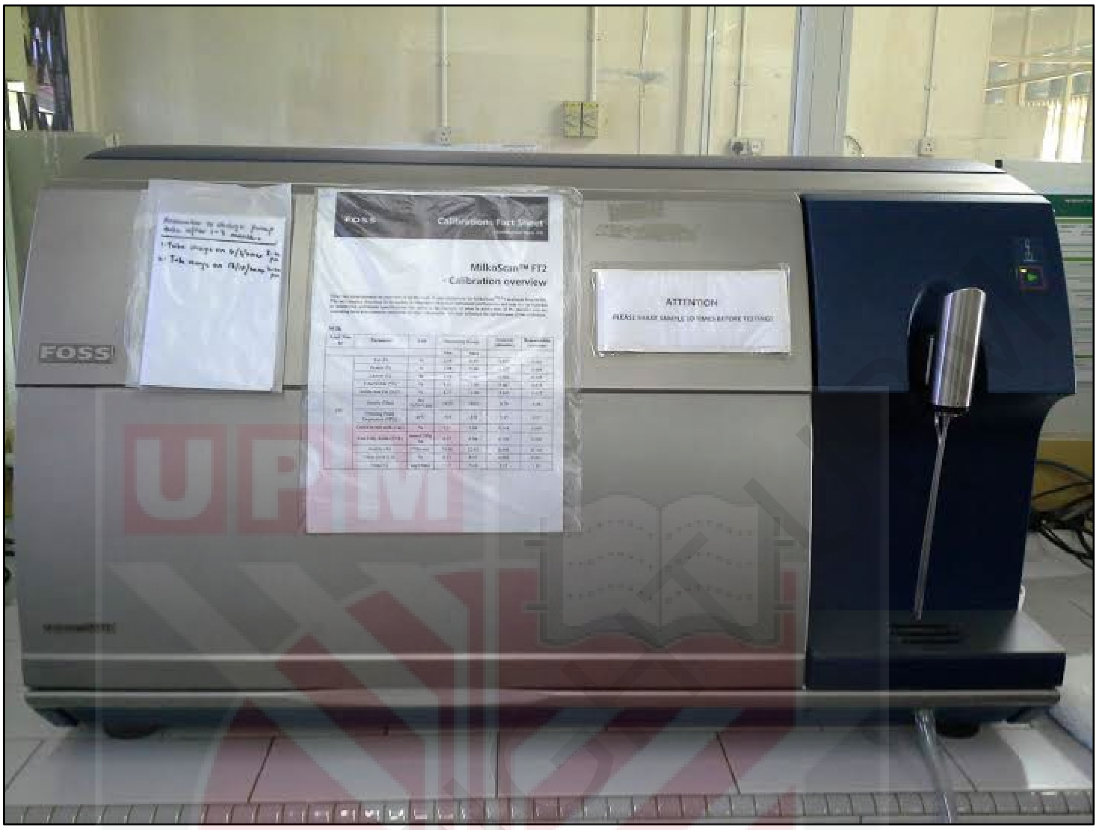
FOSS Fossomatic^{TM} Minor – Somatic Cell Count Analysis



FOSS Fossomatic^{TM} Minor – Somatic Cell Count Analysis

APPENDIX E

FOSS Milkoscan^{TM} FT2 – Milk composition Analysis



FOSS Milkoscan^{TM} FT2 – Milk composition Analysis

FOSS Milkoscan^{TM} FT2 – Milk composition Analysis