



UNIVERSITI PUTRA MALAYSIA

***MILK COMPOSITIONS OF DAIRY GOATS WITH AND WITHOUT
SUBCLINICAL INTRAMAMMARY INFECTIONS***

IFFAH NADZIRAH BT ABD RAZAK

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MILK COMPOSITIONS OF DAIRY GOATS WITH AND WITHOUT
SUBCLINICAL INTRAMAMMARY INFECTIONS.

IFFAH NADZIRAH BT ABD RAZAK

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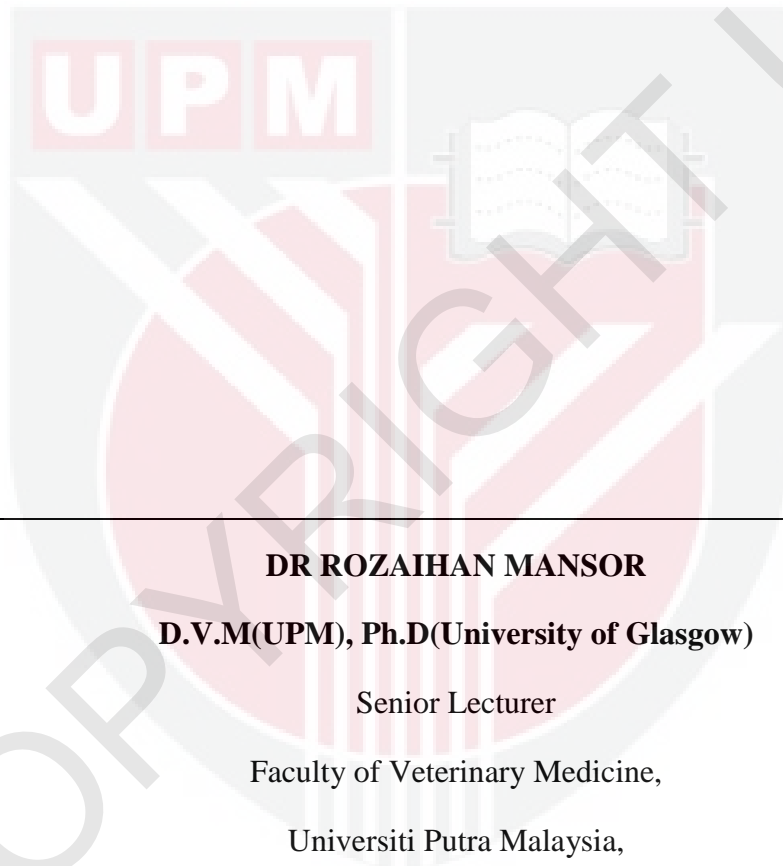
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It is hereby that we have read this project title “Milk composition of dairy goats with and without subclinical intra mammary infections”, by Iffah Nadzirah Bt Abd Razak and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment for course VPD 4999- Final Year Project.



DR ROZAIHAN MANSOR

D.V.M(UPM), Ph.D(University of Glasgow)

Senior Lecturer

Faculty of Veterinary Medicine,

Universiti Putra Malaysia,

Serdang, Selangor Darul Ehsan.



PROF.DR.ABDUL AZIZ SAHAREE

BVSc. A.H(Bombay), BVSc (Melbourne), MSc(Edinburgh), Ph.D(UPM)

Professor

Faculty of Veterinary Medicine,

Universiti Putra Malaysia,

Serdang, Selangor Darul Ehsan.



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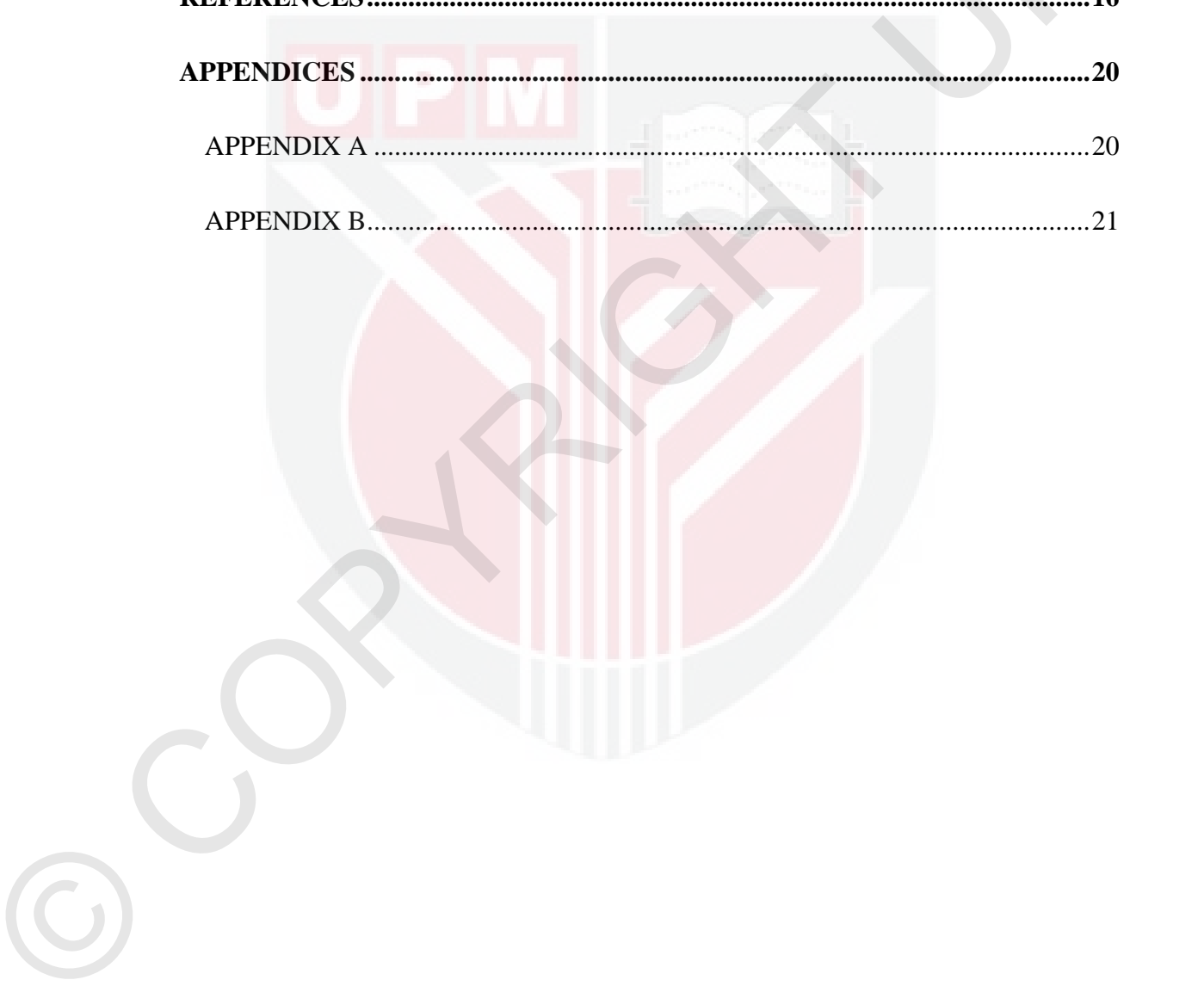
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LIST OF ABBREVIATIONS

Intramammary infections **IMI**

Somatic cell counts **SCC**

California Mastitis Test **CMT**

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ABSTRAK

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

KOMPOSISI SUSU KAMBING TENUSU DENGAN JANGKITAN INTRAMAMMARI DAN TANPA JANGKITAN INTRAMAMMARI.

Oleh

Iffah Nadzirah Binti Abd Razak

2015

Penyelia : Dr Rozaihan Mansor

Jangkitan Intramammari (IMI) menjejaskan komposisi susu seterusnya mengurangkan nilai kebersihan dan kualiti susu . Kajian ini telah dijalankan untuk membandingkan parameter komposisi susu kambing tenusu dengan dan tanpa IMI subklinikal , untuk menentukan kesan subklinikal IMI kepada komposisi susu dan juga untuk menentukan hubungan kiraan sel somatik (SCC) pada komposisi susu . Sejumlah dua puluh kambing Saanen dari salah satu Ladang Angkat Fakulti Perubatan Veterinar , Universiti Putra Malaysia (FPV , UPM) telah terlibat dalam kajian ini dan Carlifonia Matitis Test (CMT) telah digunakan untuk mengenal pasti

kambing tenusu yang dijangkiti dan tidak dijangkiti . Parameter komposisi susu termasuk lemak , protein , kasein , laktosa , jumlah pepejal , bukan lemak pepejal , keasidan , asid lemak bebas telah dianalisis dengan menggunakan FOSS Milkoscan™ FT2 . Tiada perbezaan yang signifikan ($p > 0.05$) didapati untuk semua parameter komposisi susu antara kambing tenusu yang dijangkiti dan tidak dijangkiti, bagaimanapun , terdapat perbezaan yang signifikan ($p < 0.05$) pada SCC antara kambing tenusu di Ladang Angkat FPV , UPM yang dijangkiti dan tidak dijangkiti . Di samping itu, pekali korelasi antara SCC dengan semua parameter komposisi susu didapati rendah ($R < 0.5$) . Kesemua keputusan ini berbeza daripada kajian-kajian lain sebelum ini yang menunjukkan perubahan ketara dalam komposisi susu semasa subklinikal mastitis dan hubungkait antara SCC dan parameter komposisi susu . Ia boleh disimpulkan bahawa beberapa faktor seperti saiz sampel , peringkat laktasi dan baka kambing boleh menyebabkan variasi dalam keputusan oleh itu tiada satu pun parameter komposisi susu sesuai dijadikan sebagai penunjuk kepada jangkitan intra mammari subklinikal.

Kata Kunci: kambing tenusu , komposisi susu , jangkitan subklinikal intramammari , kiraan sel somatik

ABSTRACT

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

MILK COMPOSITION OF DAIRY GOATS WITH AND WITHOUT SUBCLINICAL INTRAMAMMARY INFECTIONS

By

Iffah Nadzirah Binti Abd Razak

2015

Supervisor: Dr. Rozaihan Mansor

Intramammary infections (IMI) affect the composition of milk thus lowering the hygienic value and quality of the milk. This study was carried out to compare milk composition parameters of dairy goats with and without the subclinical IMI, to determine the effect of subclinical IMI on the milk composition and also to

determine the relationship of somatic cell count (SCC) on the milk compositions. A total 20 Saanen goats from one of Ladang Angkat of Faculty of Veterinary Medicine, University of Putra Malaysia (FPV,UPM) was involved in this study and Carlifonia Matitis Test (CMT) was used to identify subclinically infected and uninfected does. The milk composition parameters such as fat , protein, casein, lactose, total solid, solid non fat, acidity, free fatty acid were analysed using FOSS Milkoscan™ FT2. However, no significant difference($p>0.05$) was found for all the milk composition parameteres between subclinically intramammary infected does with the uninfected does, however, a significant difference ($p<0.05$) of the SCC between subclinically infected and uninfected of dairy goats at Ladang Angkat FPV, UPM was found. In addition, the correlation coefficient between SCC and all the milk composition parameters was found to be low($R<0.5$).These results differ from other previous studies that showed significant changes in the milk composition during subclinical mastitis and strong correlation between SCC and milk composition parameters.It can be concluded that no significant different of milk composition of subclinically infected and uninfected does, no correlation between SCC and milk composition and no effect of subclinical mastitis on the milk composition thus none of the milk composition parameter is suitable to indicates subclinical IMI.

Keywords: Dairy goats, milk compositions, subclinical intramammary infection, somatic cell count

1.0 INTRODUCTION

In Malaysia, there is a slow but increasing in the demand of goat's milk by increasing in the society affluence and also traditional beliefs on the added health benefits of goat milk (Sithambaram& Nizam, 2013). Thus, milk yield and the milk quality must be increased in order to meet the demand.

However, disease challenges like mastitis reduce the milk yield, the hygienic value as well as quality of the milk. This will affect the suitability of milk to be processed and the quality of its products. Subclinical IMI does not cause visible changes in the milk or udder and make it more economically important than clinical mastitis with a higher prevalence.

Subclinical IMI causing health hazards to the public since no visible changes of milk could be seen, thus the milk of infected animals can be mixed in the bulk tank which will eventually become the main source of enterotoxigenic from causative agent such as *E.coli*. (Hassan, 2013). Some strains of the *E.coli* that produce enterotoxin were also found to be resistant toward heat (Hassan(2013); Abera et at.,(2010). This study was carried out to compare milk composition parameters of dairy goats with and without the subclinical IMI, to determine the effect of subclinical IMI on the milk composition and also to determine the relationship of somatic cell count (SCC) on the milk composition.

2.0 LITERATURE REVIEW

2.1 Intramammary Infections in Goats

Intramammary infection or mastitis is the inflammation of the mammary gland or udder due to infectious or environment pathogens. Mastitis can be classified into subclinical mastitis and clinical mastitis. Clinical mastitis is characterized by pathological changes of the udder(necrosis and redness) at one or both udder as well as abnormalities of milk(flakes or clots presence in milk) and other symptoms like anorexia and fever(Conteras et al., 2007). However, in subclinical mastitis (SCM) cases, no evidence of clinical abnormalities and apparently normal milk were found but positive in bacteriological culture with increased in the somatic cell count. Although there was no apparent changes in both milk and udder of goats with subclinical mastitis, it may progress to clinical mastitis if left untreated, This, represent a constant risk of infection for the whole herds which make SCM among most important infectious diseases of dairy goats (Stuhr & Aulrich, 2011) .

2.2Milk composition

The mammary gland secretion which known as the milk contains oraganic and inorganic components like fat, protein, lactose, casein, solid non fat, free fatty acid and etc. The composition of the milk is determined by the regulation of the blood-udder barrier between the blood and the parenchyma. Meanwhile, the health status of the udder influence the integrity of blood udder barrier and thus during mastitis, the integrity of the blood udder barrier will be affected and eventually altered the milk composition. (Stuhr & Aulrich, 2011; Mungatana et al.,2011).

2.3 Somatic Cell count (SCC)

Somatic cell count (SCC) has been used as one of the methods for diagnosis of udder health in cow; however, it is not an established marker for subclinical IMI in dairy goats since factors like parity, stage of lactation, estrus, and breed contribute to changes of SCC in milk of dairy goats (Stuhr and Aulrich, 2011). Different studies have shown that goat milk has significantly higher SCC than cow's milk due to the apocrine secretion of goat's milk compared to cow's milk with a merocrine secretion. Apocrine secretion sheds a large number of cytoplasmic particles (Robertson and Muller, 2005). These non-cytoplasmic cells have no deoxyribonucleic acid (DNA) or nucleus like leucocytes do, thus contributing to a higher SCC in goat's milk. Due to the large number of non-DNA-containing particles in goat's milk, thus SCC counting methods that are based on the presence of DNA such as FOSSOMATIC and CMT will give lower counts compared to direct microscopic procedures (Robertson and Muller, 2005).

2.4 California Mastitis Test (CMT)

The principle of CMT is based on the reagent that binds to cellular DNA and destroys the membrane, thus increasing the milk viscosity depending on the amount of cells. Thus, we can roughly estimate the number of somatic cells in the milk sample (Schaereen and Maurer, 2006; Stuhr and Aulrich, 2011). CMT is also influenced by factors that cause variations of SCC; thus, CMT level correlates well with SCC in goat's milk (Schaereen and Maurer, 2006).

3.0 MATERIALS AND METHODS

3.1 Animal selection

Twenty Saanen does around 3-4 years old at 60-120 days of lactation were used in this study. The does were selected based on the California Mastitis Test (CMT) results for detection of subclinical mastitis. The animal with positive CMT results of score more than 1+ but without any pathological changes in udder and normal milk appearance was chosen for group with subclinical IMI (n=10), while the does with negative CMT results of score less than 1+ was chosen for group without subclinical IMI (n=10).

3.2 CMT

CMT was done by using CMT reagent and a CMT paddle. The udder and teat was cleaned prior to the test. Approximately about 2 ml of milk from each quarter was collected in clean CMT paddle that has four shallow cups marked A, B, C, and D (help) identify the individual quarter from which the milk was obtained). An equal amount of CMT reagent was added onto each cup in the paddle. The paddle was rotated in circular motion and the results were read within 10 seconds. Look if any gel formation and the viscosity. The results were read as negative (0), Trace, and positive (1+,2+,3+) as shown in Table 1.

Table 1 :Interpretation of CMT scores in goat's milk (Escobar, 2002)

CMT Score	Description of Reaction Between CMT Reagent and milk	Estimated number of white blood cells per ml
0	No reaction	Below 200,000
Trace	Slight slime, tends to disappear with continued swirling	150,000 to 500,000
1	Distinct slime but without gel	400,000 to 1,500,000
2	Immediate gel formation; moves as a mass during swirling	800,000 to 5,000,000
3	Gel develops a convex surface and adheres to the bottom of the cup	Over 5,000,000

3.3Milk Sampling

The teat was first stripped to discard the first stream of milk first before it was cleaned. Approximately about 50ml of milk were collected aseptically in individual Whirl-pak® bag and the milk samples were refrigerated at -20°C for further analyses.

3.4Milk composition Analyses

FOSS Milkoscan FT2™ was used for milk composition (fat, protein, lactose, total solid, solid non fat, acidity, casein) analyses. Fossomatic Minor™ somatic cell counter was use to count the somatic cell in the milk.

3.5 Data Analysis

Data analysis was done using IBM SPSS version 22. Independent sample T-test was used to compare the mean of milk composition and SCC between the two groups. Pearson correlation was used to know the correlation between SCC and milk composition.



4.0 RESULTS

The fat, protein, lactose, casein, total solid, acidity and total solid concentrations ($p > 0.05$) did not differ between the does with and without subclinical IMI (Table 1) however, the SCC of does with subclinical IMI was significantly higher ($p < 0.05$) as compared with does without subclinical IMI (Table 2).

The correlation between SCC with all component was not poor with the correlation coefficient was less than 0.5 ($R < 0.5$). Therefore, it can be concluded that SCC was negatively correlated with other components of milk composition as shown in Table 3.

Table 2: Milk composition and somatic cell count of dairy goats with and without subclinical IMI (Mean±SE)

Parameter	Without subclinical IMI Mean±SE	With subclinical IMI Mean±SE	P value*
SCC ± se (x10 ³)	1155.0±221.12	2250.2±298.461	0.009*
Fat (%)	4.48±0.277	4.90±0.447	0.432
Protein (%)	2.76±0.060	2.84±0.075	0.411
Lactose (%)	4.03±0.038	3.96±0.026	0.149
Casein (%)	2.09±0.039	2.12±0.071	0.626
SNF (%)	7.73±0.072	7.77±0.093	0.731
FFA (%)	0.85±0.085	0.70±0.048	0.143
Acidity (%)	14.49±0.785	14.56±0.643	0.944
Total solid (%)	11.93±0.276	12.33±0.485	0.481

*significant at p value < 0.05

Table 3: Correlation of SCC and component of milk composition

Parameters	SCC	Fat	Protein	Lactose	Casein	SNF	FFA	Acidity	Total solid
Fat	0.365								
Protein	0.383	0.283							
Lactose	-0.123	-0.320	-0.130						
Casein	0.333	0.331	0.900**	-0.321					
SNF	0.175	0.390	0.784**	-0.050	0.760**				
FFA	-0.419	0.148	-0.565**	-0.072	-0.452*	-0.197			
Acidity	0.048	0.339	0.162	-0.663**	0.404	0.119	0.295		
Total solid	0.372	0.987**	0.385	-0.287	0.333	0.515*	0.167	0.350	

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

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

5.0 DISCUSSION

The milk from infected goats will cause increase in the lipase activity that result in milk fat breakdown and the release of free fatty acid that cause milk off flavour(Nagwa et al., 2000; Uallah et al.,2005). Thus during subclinical IMI, there will be reduced in milk fat and increase in free fatty acid. However, the results of this current study show no significant different in between the 2 groups of dairy goats in milk fat and free fatty acid composition.

This results was in accordance with a study by Leitner et al.,(2004a), who demonstrated that there was no difference of between infected halves($3.88\% \pm 0.12$) and uninfected halves ($3.89\% \pm 0.11$) in a sample of 25 crossbred goats. Another study using 10 different herds also demonstrated that no significant difference on fat composition of infected(3.75%) and uninfected goats(4.2%)(Leitner et al., (2004b)).Min et al.(2007) also confirmed the results with 35 mixed age Alpine goats that there was no significant interaction between milk fat and infection status while a study by Hassan (2013) shows disagreement with significantly reduced in milk fat compositions of 45 crossbreeds goats with subclinical IMI. This is because many factors such as type and quantity of feed, breed, stage of lactation, lactation numbers and seasonal changes have effect on the milk fat composition that make variation in the results from different studies.

This is supported in one study by Souza et al.,(2009) revealed that all the milk components from 7 herds were significantly affected by season and herd. A study by Ying et al.(2002) showed significant results of between early and late lactational stages. Thus, it can be concluded that milk fat is not a suitable parameters to indicate IMI in dairy goats.

According to Stuhr & Aulrich,(2011)milk protein is a relatively an autonomous parameter that can be affected mainly by the type of feeding. Milk proteins are mainly composed of caseins and various enzymes and the normal physiological range of milk protein according to Souza et al.(2009) was 2.85% to 3.00%. The results of this current study were not within the range but shows increased in milk protein in infected goats compared to uninfected goats ($2.76\pm 0.06\%$ vs $2.84\pm 0.08\%$).

However, the result was not statistically significant. This was in accordance to Leitner et al. (2004a) whom found an increased of milk protein in infected udder halves ($3.42\pm 0.05\%$ vs $3.50\pm 0.05\%$) but not statistically different. Another study by Leitner et al. (2004b) with 10 herds of mix breed goats with 50 goats in each herds demonstrated a significantly higher protein in infected udder than uninfected udder (3.91% vs 3.99%).Ying et al.(2002) demonstrated a significant changes in milk protein during early lactational stage but not with late lactational stage. Meanwhile, Min et al. (2007) failed to measure the significant effect of IMI on the concentration of protein. Factors like feeding, type of feed and lactational stage affect the protein composition in milk which explained the difference of milk protein concentration in

many studies. Thus, it can be concluded that protein also not a suitable indicator for IMI in dairy goats.

Lactose is synthesized by glucose and galactose in the gland cell of udder and, during IMI, this physiological process was partially disabled which result in reduced lactose composition of milk in infected animal(Stuhr and Aulrich, 2011). The lactose content in goat's milk was influenced by many factors such as breed, feeding, lactation number and age and the average range of lactose in milk is from 3.8% to 4.6% (Jendretzke, 2009). In this current study, the lactose concentration was found to be decreased in infected goats than uninfected goats with IMI (3.96 ± 0.026 % vs 4.03 ± 0.038 %) but not significantly different. In a study with 25 crossbreeds goats, Leitner et al. (2004a) found a significantly lower lactose concentration in infected as compared to uninfected udder (4.17 ± 0.13 % vs 4.70 ± 0.10 %). In another study by Leitner et al. (2004b) with 10 herds of goats also demonstrated that lactose concentration was significantly lower in infected than uninfected udder (4.72 % vs 4.96 %). Min et al. (2007) demonstrated that concentration of lactose in milk was tendentially lower in infected udder at early and mid lactation and during the late lactation, the concentration of lactose reduced with and without the subclinical IMI. Thus lactational stage can affect the milk lactose concentration. Jendretzke (2009) did a study comparing the CMT scores with the lactose concentration in goat's milk at early lactation and late lactation and it showed significant average decrease of 0.82% of lactose in late lactation(4.26 %) as compared to early lactation(5.08 %). In addition, a slight decrease of lactose can only be seen in CMT

score of 3+. Furthermore, the lactose content of different breeds and age group were also investigated and it showed that the lactose content also varied among the six different breeds with a slight different of lactose content between five different age groups. Thus, we can conclude that lactose not a suitable parameters to indicate IMI in dairy goats.

Other component of milk composition like casein, solid non fat(SNF), total solid, acidity, free fatty acids showed no significant different in this current study which was in accordance with many previous studies of dairy goats. However, Hassan (2013) found that SNF was significantly lower in concentration in cow's milk infected with subclinical IMI but not in the goats and ewes milk.

Generally the goat's milk has higher SCC than cow's milk due to apocrine secretion as mentioned before. The SCC of dairy goats with subclinical IMI was found to be significantly higher than the dairy goats without subclinical. An increase in SCC during infections is due to increase in shedding of leucocytes and other inflammatory cell in milk as a defence mechanism. However the normal accepted level has not been established and the SCC in bulk tank goat's milk varies greatly between countries (Robertson and Muller, 2005). In United States the legal limit of SCC is not exceed 1 million cell/ml while in European countries such as Italy, Spain and France the SCC were 1753000 cells /ml, 1600000 cells /ml and range of between 1200000 to 1500000 cells /ml respectively (Robertson and Muller, 2005)

SCC can be used as an indicator of IMI , however there are uncertainties of the legal limit of SCC in goat's milk. The somatic cell counter such as FOSSOMATIC which employed a special stain can be used to count somatic cell rather than by using direct microscopic method(Robertson and Muller, 2005).CMT on the other hand, can be used as a screening test for subclinical IMI since it based on SCC and the CMT reagent bind to DNA and destroying the cytoplasmic membrane that will cause increase in the viscosity of the milk sample.

The current study showed no correlation between SCC and all the components of milk composition with correlation coefficient $R < 0.5$. SCC was found to be positively correlated with protein percentage (Ying et al.,2002), fat percentage, pH(Hamed et al.,1993)but negatively correlated with casein, acidity percentage, total solid, and lactose(Hamed et al.,1993) In a study with different breeds of goats such as Saanen, Toggenburg and Anglo- Nubian, SCC was not significantly correlated with all the component of milk composition and this in accordance to this study in which Saanen breed goats was used.

6.0 CONCLUSION AND RECOMMENDATIONS

The composition of goat's milk were not affected by subclinical IMI and other factors like lactational stages, age, and type of feed can affect the composition of goat's milk. It was also found that the SCC was poorly correlated with milk composition parameters.. Thus it can be concluded that none of the component of milk composition could be a suitable indicator of IMI in dairy goats.

The recommendation for further study is to include larger sample size involving different herds of goats in getting better statistically significant results.

Considerations on other factors like the type of feed, breed, age, lactational stages can be included in the study which also affect the composition of the milk

Further study on other indicator of IMI in dairy goats for example the enzyme such as lactoferrin, beta-glucoronidase, and lactate dehydrogenase could also be done in order to determine the suitable indicator for IMI especially subclinical IMI other than gold standard method which is the bacteria isolation and identifications.

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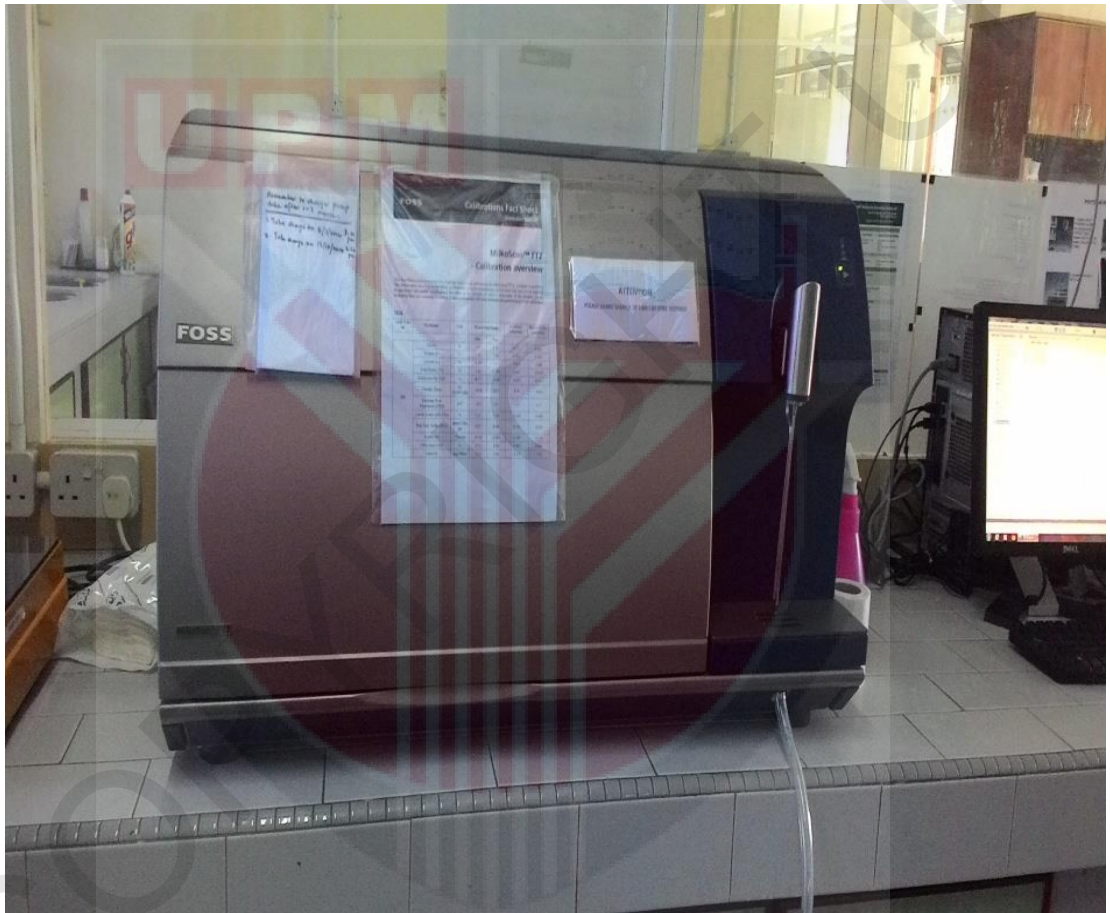
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APPENDICES**APPENDIX A**

FOSS Milkoscan FT2- Milk composition Analysis

APPENDIX B



FOSSOMATIC MINOR- Somatic Cell Counter