UNIVERSITI PUTRA MALAYSIA

BUFFALO (*Bubalus bubalis*) MILK QUALITY EVALUATION FROM DIFFERENT FARM IN THE STATE OF KEDAH DARUL AMAN

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BUFFALO (*Bubalus bubalis*) MILK QUALITY EVALUATION FROM DIFFERENT FARM IN THE STATE OF KEDAH DARUL AMAN

ABDUL QHANI BIN DALI

A project paper submitted to the Faculty of Veterinary Medicine, University Putra Malaysia in partial fulfilment of the requirement for the DEGREE OF DOCTOR OF VETERINARY MEDICINE Universiti Putra Malaysia, Serdang, Selangor Darul Ehsan.

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Buffalo (*Bubalus bubalis*) Milk Quality Evaluation from Different Farm in the state of Kedah Darul Aman.", by Abdul Qhani bin Dali and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement of the course VPD 4901 – Project.

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DEDICATIONS

To God Almighty Allah my creator

My beloved family,

Ayah

Mak

Brothers

Sisters

And Friends

Thank you for the unconditional love and endless support

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I would like to express my gratitude to Allah for giving me strength and guidance throughout the four weeks journey in completing my final year project.

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Oleh

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2018

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Kajian ini bertujuan bagi menilai kualiti susu kerbau yang dihasilkan dari beberapa ladang di negeri Kedah Darul Aman, Malaysia. Sampel susu mentah telah dikumpulkan secara rawak dari lima ladang yang menghasilkan susu kerbau menggunakan baka Murrah yang diuruskan dalam sistem semi intensif bersepadu. Ladang-ladang tersebut terletak di tiga daerah di negeri Kedah iaitu Kuala Ketil, Kulim dan Pendang. Lima parameter terpilih telah diguna pakai dalam menentukan kualiti susu kerbau iaitu takat kestabilan susu, kualiti penyimpanan, komposisi, sisa antimikrobial dan status kebersihan. Analisis takat kestabilan susu ditentukan melalui ujian alkohol 80%, manakala kualiti penyimpanan telah ditentukan menggunakan Ujian Penurunan Warna Metilena (MBRT). Analisis komposisi susu kerbau yang merangkumi penentuan kandungan lemak, protein, laktosa, pepejal tanpa lemak dan jumlah pepejal telah dijalankan dengan menggunakan Lactoscan Milk Analyzer yang ditentukur khas untuk susu kerbau. Analisis untuk penentuan sisa antimikrobial di

dalam susu telah dijalankan menggunakan teknik Kit Ujian Delvo. Penghitungan mikrob dijalankan menggunakan metodologi hitungan cawan (TPC). Kedua-dua ujian alcohol 80% dan MBRT menunjukkan keputusan negatif, menandakan kestabilan susu dan beban mikrobial berada pada tahap yang baik. Analisis komponen kimia serta komposisi susu menunjukkan hasil lemak, protein, laktosa, kandungan pepejal bukan pepejal dan pepejal agak rendah berbanding dengan piawaian untuk susu kerbau dan juga artikel-artikel yang diterbitkan. Tiada sisa antibiotik yang dijumpai dalam keseluruhan sampel dan juga hasil penghitungan mikrobial tidak melebihi nilai yang ditetapkan dalam Malaysia Food Regulation (1985). Oleh itu, kajian ini menyimpulkan bahawa susu kerbau yang dihasilkan di negeri Kedah mempunyai kualiti yang baik.

Kata kunci: Susu kerbau, parameter kualiti susu, kerbau (*Bubalus bubalis*), Kedah

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 – Project.

BUFFALO (*Bubalus bubalis*) MILK QUALITY EVALUATION FROM DIFFERENT FARM IN THE STATE OF KEDAH DARUL AMAN

By

Abdul Qhani bin Dali

2018

Supervisor: Prof. Dr. Md. Zuki bin Abu Bakar

Faculty: Faculty of Veterinary Medicine

The present research aimed to evaluate the quality of buffalo milk produced from different farm in Kedah. Raw milk samples were randomly collected from five buffalo farms of Murrah breed managed in integrated semi-intensive system, located in three district of Kedah which are Kuala Ketil, Kulim and Pendang. Five selected parameters were used in determination of the buffalo milk quality which are the milk stability, keeping quality, composition, antimicrobial residue and sanitary and hygienic status. Analysis of milk stability and keeping quality was determined through alcohol test and Methylene Blue Reduction Test (MBRT) respectively. Analysis of the buffalo milk composition such as fat, protein, lactose, total solids and solid non-fat were carried out using Lactoscan Milk Analyzer calibrated for buffalo milk. Analysis of antimicrobial residue were carried out using Delvo Test Kit technique. Microbial enumeration was carried out using Total Plate Count technique in order to determine the sanitary and

hygienic status of the milk. Both alcohol test and MBRT reveal negative results, indicating good milk stability and keeping quality. The analysis of the chemical components as well as composition of the milk shows the result of fat, protein, lactose, total solid and solid non-fat content were quite low than the standard value for buffalo milk and also published articles. No antibiotic residues were found in the totality of the sample and also the microbial enumeration result does not exceed the value set in Malaysia Food Regulations (1985). Thus, this study concluded that the buffalo milk produced in the state of Kedah are of good quality.

Keywords: Buffalo milk, milk quality parameter, buffalo (*Bubalus bubalis*), Kedah,

INTRODUCTION

Milk can be defined as a white liquid produced by the mammary gland of mammalian and it is considered as an almost complete food for human diet (Khedkar et al., 2016). It is the most important source of nutrition for young mammals before they grow up as all of the essential nutrients that can be found in milk are protein, fat, lactose, vitamins, and mineral matter for normal growth and performing different functions for the body systems (Khedkar et al., 2016). Usually for human consumption, especially in Malaysia, most of the population tend to consume more cow and goat milk. Not to forget the mother's milk, these two types of milk often being the most preferable choice of replacement for mother's milk. However, buffalo milk also has the potential to be one of the popular milk for consumption in Malaysia.

The domesticated water buffalo are scientifically known as *Bubalus bubalis* (Abd El-Salam and El-Shibiny, 2011). Water buffalo can be further divided into two sub-species, which are the river type and the swamp type (Shaista et al., 2017). In Malaysia, the water buffalo or simply known as Malaysian buffalo is a swamp type and can be found in the western peninsular of Malaysia (Mason, and I.L, 1996). Buffalo can be a source of draft power, transportation, on-farm manure, meat, milk and livelihood of the farmers. Buffalo have remarkable potential in utilizing the agricultural crop-residue and by products, due to the fact that they have been raised by the rural farmers in the harsh environment (Wanapat and Kang, 2013).

Recently, buffalo milk production in Asia represents 96.78% of the total volumes of world's buffalo milk which is estimated at 89.2 Million tons. Production in South and

Southwest of Asia, are dominated by India and Pakistan, in which 93.17 % of world buffalo milk production come from these two countries (FAO, 2010). China is among the largest producer countries of buffalo milk, with both buffalo herds and buffalo milk production listed third worldwide in 2004, after those of India and Pakistan (FAO, 2004). As stated by Wanapat and Kang (2013) the average annual growth rate in buffalo milk production in the whole of Asia between the years 1998 to 2008 was 4.39%. Currently, Myanmar entitled as the highest buffalo milk producer among the South East Asia countries due to the reason that it had the highest population of dairy buffalo (Hlaing, 2001). In other South East Asia countries, the buffalo milk production is currently very low as the farmers tend to use their animals mainly for work only (Wanapat and Kang, 2013).

Generally, buffalo milk is among the milk that having high nutritional content characterized by high level of solid constituents (Bailone et al., 2017). Wanapat and Kang (2013) stated that buffalo milk contains higher composition of total solids, fat, protein and lactose as compared to cow milk. This is also being reported by Araújo et al. (2011), saying that 'the great advantage of buffalo milk in relation to cow milk (*Bos taurus*) is precisely its high amount of fat, protein, lactose, dry extract, and total solids'. Buffalo milk is much healthier as it is higher in saturated fatty acids (Wanaphat and Kang, 2013). This is further supported by studies done by Van Nieuwenhove et al. (2004) and he concluded that, buffalo milk and cheese are a good source of Conjugated Linoleic Acid (CLA) for human nutrition. Swamp buffalo milk has even higher fat (7.9%), protein (4.2%), Calcium (264.0 mg/100 g) and Cholesterol (0.65 mg/g).

There are many parameters that can be used as an indicator to define a quality milk. Some of the popular milk quality parameter being used in most developed countries are the Somatic Cell Count (SCC) and the total plate count (TPC) and the evaluation of these two parameters for bulk milk regarded as an internationally recognized method to establish milk quality as well as the udder health status of the cows in the herd (Cicconi-Hogan, 2013). Other milk quality parameter such as composition and physical-chemical characteristic can also be used (Pasquini et al., 2017). In this study, five parameters were chosen based on their availability to indicate quality of buffalo milk, which are the milk stability, keeping quality, composition, antimicrobial residue and sanitary and hygienic status. Analysis of milk stability was determined through alcohol test, while keeping quality was determined through Methylene Blue Reduction Test (MBRT). Analysis of the buffalo milk composition which include the determination of fat, protein, lactose, total solids and solid non-fat were carried out using Lactoscan Milk Analyzer calibrated for buffalo milk. Residues for antimicrobial analysis were carried out using Delvo Test Kit technique. Microbial enumeration was carried out using Total Plate Count technique in order to determine the sanitary and hygienic status of the milk. Milk analysis can be used to detect the presence of antimicrobial drugs used in the treatment of cattle infection (Bailone et al., 2017).

In short, buffalo milk actually possesses significant advantage to be developed in our country. However, since the quality evaluation of the buffalo milk in Malaysia particularly are less studied, therefore, as a beginning, this study aim is to evaluate the quality of buffalo milk being produced from different buffalo farms in the state of Kedah. We hope that, the information from this study could be useful in the future,

when the consumption of buffalo milk and by product becoming more popular and important in the country and in the region.

Hypothesis

The buffalo (*Bubalus bubalis*) milk from different farm in the state of Kedah are of good quality.

Objectives

This study was conducted to evaluate the quality of buffalo milk produced from different farm in the state of Kedah.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk

According to Khedkar et al. (2016) milk can be defined as an almost complete food for human diet. Kanwal et al. (2004) defined milk as a substance created to feed mammalian infant that includes from man to even whales. The domestication of animal including cattle, buffaloes, goat and even camel by the ancient human for milk purposed have been dated back from 6000-8000 B.C (Eddleman, 1999). Being the first food of the newly born human being and other mammals, it is a food that contains all the nutrients required for the newly born baby, pregnant mothers, patients, and even the old age people (Khedkar et al., 2016).

Khedkar et al. (2016) also said that milk and milk products have played a key role throughout the development of human civilization and supply most of the essential nutrients in significant amounts than any other single food, as it is very essential for the growth and development of a newly born child. All of the essential nutrients that can be found in milk are protein, fat, lactose, vitamins, and mineral matter for normal growth and performing different functions for the body systems (Khedkar et al., 2016). Not only it is the most important food during early childhood, but also, in one form or another, it continues to be used for normal diet throughout the life span (Khedkar et al., 2016).

It is also the most versatile of all the animal-desired food commodities, and it is a component of the diets of many physical forms like cheese, yogurt, ice cream, ghee, milk powders, and many other forms of fluid milk (Khedkar et al., 2016). Milk is also a very complex substance as they naturally possess many physical and chemical components and these components are the same throughout different species but in varying amount (Roadhouse, 1950). Milk is a very complex substance as they naturally contain abundant chemical and physical components and every milk of different species having the same kind of constituents but in different amounts, because this component can be affected by several factors such as genetic, environmental factor and stage of lactation (Kanwal et al., 2004)

2.2 Buffalo Milk Production

According to Pasquini et al. (2017) buffalo milk is the second most consumed milk worldwide after cow milk and the biggest production of buffalo milk came from India and Pakistan as they produce more than 91% of the total buffalo milk in the world. This is also supported by the data from FAO in 2010 as buffalo milk production in Asia represents 96.78% of the total volume of the world's buffalo milk of 89.2 million tons. China is among the largest producer countries of buffalo milk, with both buffalo herds and buffalo milk production listed third worldwide in 2004, after those of India and Pakistan (FAO, 2004). As stated by Wanapat and Kang (2013) the average annual growth rate in buffalo milk production in the whole of Asia between the years 1998 to 2008 was 4.39%. Production in South and Southwest Asia, primarily from India and Pakistan, contributed a hefty 93.17% (FAO, 2010).

Currently, Myanmar entitled as the highest buffalo milk producer among the South East Asia countries due to the reason that it had the highest population of dairy buffalo (Hlaing, 2001). In other South East Asia countries, the buffalo milk production is currently very low as the farmers tend to use their animals mainly for work and only a small portion of resulting crossbreds between the dairy buffalo breeds with the existing swamp buffalo population and also of newly introduced riverine buffaloes are being fully utilized for milk production purposes (Wanapat and Kang, 2013)

In Europe, the countries that produce the largest quantities of buffalo milk are Italy, Turkey, Bulgaria and Greece (FAOSTAT 2014). Cazacu et al. (2014) said that, the buffalo milk market is still considered as an emerging sector. The dairy industry produces many products based on the use of buffalo milk such as pasteurized or concentrated milk, butter, heat-desiccated dairy products, heat-acid coagulated dairy products, yogurt, ice-cream, dehydrated milk products and cheeses (Cazacu et al. 2014). The high quality and manufacturing of buffalo-based dairy by products are being contributed by the high fat and protein in buffalo milk (Tonhati, 2000). Not only that the buffalo milk is being utilized and transformed into various valuable products, it is also much more preferred by the consumer for its rich in nutritional value (Khedkar et al., 2016).

2.3 Buffalo (*Bubalus bubalis*)

Buffaloes can be classified taxonomically to the order of Artiodactyla, suborder Ruminantia, family Bovidae, tribe Bovini (Bhattacharya, 1964). Swamp Buffalo or locally known in Bahasa as “Kerbau Sawah” is the indigenous breed of buffalo in

Malaysia (Malaysia Livestock Breeding Policy, 2013). FAO (2005) stated that the domestic water buffalo is commonly found in Malaysia and can be classified into the River and Swamp types. According to Khedkar et al. (2016) there are two main types of buffaloes in the world: (1) riverine buffaloes (*Bubalus bubalis*) and (2) swamp buffaloes (*B. carabanesis*), whereas the third type known as Mediterranean buffaloes evolved from these two major types. However, Ianuzzi and Di Medeo (1995) stated that, the buffalo are classified into two main species, which are the Asian buffalo (*Bubalus bubalis*) and the African buffalo (*Syncerus caffer*). Buchholz (1990) then further classified the African buffalo into two subspecies which are the forest buffalo (*Syncerus caffer nanus*) and the cape buffalo (*Syncerus caffer caffer*).

The river buffalo are populated in India continent while the swamp type is populated in the East and Southeast Asia (SEA) (Cruz, 2010). There are about 170, 000 of total buffalo population in Malaysia in the year 1998 and they can be found mostly concentrated in the rice growing states of Kelantan, Terengganu, Kedah and Pahang in West Malaysia (FAO, 2005). Recent data from Annual Livestock Statistics, Department of Veterinary Services Malaysia shown that, the population of buffalo in the year 2010 are 125, 175 (Department of Veterinary Services, 2010). In Malaysia there has been an alarming decline in the buffalo population over the past two decades with an average rate of population decline of 1.2 percent per year (FAO, 2005). Displacement of buffalo by machinery for draught power in the rice fields, a low reproduction rate and a high extraction rate are said to be the reason for such a decline (FAO, 2005). The Malaysia Livestock Breeding Policy (2013) stated that the reason for buffalo has lost its prominence due mainly to farm mechanization and urbanization.

Due to the variability in work that they have been assigned for, such as for ploughing paddy fields, transportation and as a supply of meat during the period of 15th and 16th century, the Swamp Buffalo was considered as the most significant rural animal (Malaysia Livestock Breeding Policy, 2013). In comparison to other domestic livestock, water buffalo is generally considered a hardy animal despite the fact that they live in a hot and humid region that are preferable for the occurrence of many diseases (Michelizzi et al., 2010). However, the reason for this is not well known (Michelizzi et al., 2010). Water buffalo are less vulnerable to parasite and tick infestation due to their behavior of wallowing in muds to cool their body (FAO, 2000). Swamp buffalo is farmed mainly for meat production under an extensive production system. Some oil palm estates are using swamp buffalo as draught animals for pulling carts carrying oil palm bunches. River buffaloes were imported from the Indian subcontinent in the 1920s and are mostly found in Selangor, Perak and Kedah and raised for milk production (Jainudeen and Wan Zahari, 2000). New genetic material in the form of live river buffaloes were introduced by Department of Veterinary Services (DVS) with the importation of 150 Murrah buffaloes from India in 2010 and 170 Nili Ravi buffaloes from Pakistan in 2011 (Malaysia Livestock Breeding Policy, 2013).

There is not much emphasis on genetic improvement for buffaloes in Malaysia. In general, since a Swamp Buffalo herd never mixes with a River Buffalo herd, both are bred pure. For dairy buffalo, the farmers use river buffaloes such as the Murrah. The DVS has Murrah and Nili Ravi as dairy buffaloes. The State of Kedah is currently breeding Swamp Buffalo with imported semen from Italian Mediterranean Water Buffalo on the island of Langkawi, to produce a crossbred animal that can be used for

both milk and beef. Similarly, in Sabah, government and private farms also breed buffaloes. In 2004, Sabahmas Plantations in Lahad Datu embarked on a Swamp Buffalo breeding project with the aim to produce more draft buffaloes for pulling carts in the plantations. The Swamp buffaloes were imported from Northern Territory, Australia. The State Government of Sabah has a Buffalo Breeding and Research Center at Telupid. Swamp and Murrah buffaloes and their crossbreds are reared at the Center. Almost all buffaloes in Malaysia are bred through natural mating. Only a small percentage of them are bred through Artificial Insemination particularly in Buffalo Park, Pulau Langkawi (Malaysia Livestock Breeding Policy, 2013).

According to Malaysia Livestock Breeding Policy (2013), the River buffaloes are not indigenous to Sabah, as the early Chinese traders brought the river buffaloes to Sabah during the pre-World War II period. In 1976, a Buffalo Multiplication and Research Centre was established at Sook and the center was relocated to the present site at Telupid in 1979. In 1995, a crossbreeding program between the swamp buffalo at the center with the river buffalo through artificial insemination was initiated. However, most of the farmer in Malaysia does not practice artificial insemination in buffalo due to poor estrus identification technique (Jainudeen and Wan Zahari, 2000). The frozen semen was donated by the then Universiti Pertanian Malaysia. In 2005, the Northern Territory Government of Australia donated 5 head of crossbreed river buffalo bulls as part of the "Tender Buff" breeding program in Sabah.

The buffalo breed found in Sarawak is the swamp type (*Bubalis carabanesis*). In the 1960s the buffaloes were used in paddy land preparation for cropping rice under the Assistance to Paddy Planters Scheme. Buffaloes were actively imported in 1965 to

1968 where some 1,100 were imported annually. They are used in many rituals, ceremonies and festivals of the Bisaya, Kedayan, Malay and Lun Bawang communities of Limbang Division, such as in the Babulang Water Buffalo Racing Festival and the Ratu Babulang competition (buffalo beauty contest). In 1994, the Agriculture Department brought in 60 head of Murrah buffalo (Bubalis bubalis) and these were kept at the Batu Danau Buffalo Station in Limbang (Malaysia Livestock Breeding Policy, 2013).

The river buffalo and swamp buffalo can be differentiated based on their physical appearance, body size, biological characteristic and the chromosomes karyotype (Yue et al., 2013). Swamp buffalo is a stocky animal and prefers marshy environment while river buffalo has a massive body with curled horn and prefer clean water (Mudgal and Sethi, 1990). The river buffalo has 50 chromosomes with five pairs are submetacentric and 20 are acrocentric. The swamp buffalo has 48 chromosomes with 19 pairs are metacentric (Degrandi et al., 2014). The difference in chromosomes in these subspecies is due to the fusion between chromosomes 4 and 9 pair in the swamp buffalo genome (Moaeen, 2014). Groeneveld et al. (2010) stated that the crossbreed between swamp and river buffalo has resulted in an offspring with 49 chromosomes.

River buffalo are heavier compared to swamp buffaloes. River buffalo can weight in between 450 and 1000 kg while swamp buffalo can reach the weight of 325 and 450 kg. Swamp buffalo are mainly reared for draught purpose while river buffalo are mainly for milk production (Michelizzi et al., 2010). Jainudeen and Wan Zahari (2000) stated that most buffaloes, mainly Swamp type able to produce two offspring in every three years. There are many domesticated breeds of Indian buffaloes being used for

milk production such as Murrah, Nili-Ravi, Mehsana, Surti, Zaffarabadi or Jafarabadi Bhadawari and many more, but the most popular one is Murrah (Park, 2008).

2.4 Buffalo Milk Chemical Composition as Compared to Cow Milk

Generally, buffalo milk is among the milk that having high nutritional content characterized by high level of solid constituents (Bailone et al., 2017). The comparative composition between buffalo and cow milk are shown in Table 1. In generally, buffalo milk contains higher composition of total solids, fat, protein and lactose as compared to cow milk. This is also being reported by Araújo et al. (2011), saying that 'the great advantage of buffalo milk in relation to cow milk (*Bos taurus*) is precisely its amount of fat, protein, lactose, dry extract, and total solids'. Ahmad (2013) also stated that buffalo milk contains twice as much of fat content compared to cow milk. Under normal conditions, buffalo fat content can have an average value of between 8.3% up to 15% (Varrichio et al., 2007).

Generally, ruminant meat and milk contain various fatty acids, especially conjugated fatty acids (CLA), which propose beneficial biological activities in mammalian species (Wanapat and Kang, 2013). This is further supported by Khedkar et al. (2016) as they mention that, the CLA content of food being derived from ruminants are much higher than those from non-ruminants and milk having the higher content than meat. According to Ip et al. (1991) the cis-9, trans-11 CLA (rumenic acid, RA) has been proven to be able to decrease the incidence of cancer. The CLA components of meat and milk is greatly related to the ruminal biohydrogenation (BH) of cis-9, cis-12 C18:2 (linoleic acid, LA) and cis-9, cis-12, cis-15 C18:3 (linolenic acid, LNA) (Wanapat and

Kang, 2013). Due to this beneficial characteristic of buffalo milk, the buffalo was recently introduced in Argentina as an alternative breed, with a milk of good nutritional value and with high protein and fat content (Van Nieuwenhove et al., 2004). The influence of buffalo dairy products on lipid mice tissues was determined as both cis-9, trans-11 and trans-10 CLA, and also cis-12 CLA were incorporated into the liver, intestines and plasma of mice fed milk or cheese and the concentration of CLA was higher in tissues of mice fed cheese results clearly indicate that buffalo milk and cheese are a good source of CLA for human nutrition (Van Nieuwenhove et al., 2004).

Table 1. Typical composition of buffalo milk and cow milk.

Traits	**Buffalo**	**Cow**
Total solids (%)	16.30	13.10
Fat (%)	7.90	4.30
Protein (%)	4.20	3.60
Lactose (%)	5.00	4.80
Tocopherol (mg/g)	0.33	0.31
Cholesterol (mg/g)	0.65	3.14
Calcium (mg/g)	264.00	165.00
Phosphorus (mg/g)	268.00	213.00
Magnesium (mg/g)	30.00	23.00
Potassium (mg/g)	107.00	185.00
Sodium (mg/g)	65.00	73.00
Vitamin A (incl. Carotene) IU.	33.00	30.30
Vitamin C (mg/g)	6.70	1.90

Source: Wanapat and Kang (2013).

2.5 Parameters used in Determining Milk Quality

There are many parameters that can be used as an indicator to define a quality milk. Some of the popular milk quality parameter being used in most developed countries are the Somatic Cell Count (SCC) and the total plate count (TPC) and the evaluation of these two parameters for bulk milk regarded as an internationally recognized method to establish milk quality as well as the udder health status of the cows in the herd (Cicconi-Hogan, 2013). Hashmi & Saleem (2014) stated that, the fact that buffalo milk contains high amount of nutrient content, microorganisms are able to rapidly replicate especially in the liquid milk medium and this can result in serious effect to its commercial value. Thus, the analysis of the Total Plate Count (TPC) can be a good indicator for hygienic and sanitary status of the milk at the dairy farm level during the phases of milking, storage, and transportation (Gunasekera et al., 2003). Gargouri et al. (2013) also stated that, the TPC is able to determines the concentration of microorganisms present in the milk, thus it is a good tool for dairy hygiene and sanitary quality checkers to determine the likely adverse effect that a specific batch of milk might have on a processing plant and the overall safety of the milk. Higher milk hygiene at farm level provides the industry with better product to process resulting in products with longer shelf-life (Bailone et al., 2017).

Milk analysis can be used to detect the presence of antimicrobial drugs used in the treatment of cattle infection (Bailone et al., 2017). In particular to this, World Health Organization (WHO) and Food and Agriculture Organization (FAO) have set standards for Acceptable Daily Intake and Maximum Residues Limits (MRLs) for each antibiotic present in the milk so that the well-being of human health can be

assured. These limits apply to both the parent drug or chemicals and their resulting metabolites following the administration of a drug compound to animals. These drugs may have a cumulative effect and either be deposited or stored within the animal cells, tissues, and organs. Microbial infections in the udder will lead to detrimental effect in the milk composition, resulting in lesser suitability of the milk for human consumption and also further processing. Thus, the presence of antibiotic trace in the milk can be used as one of the parameter for milk quality (Sharif et al., 2008). There can be several reasons as to why traces of antimicrobial agent can be found in the milk, such as the herd of animal has been treated against mastitis and the farmers fail to adhere to the recommended withdrawal period of the used antimicrobial agent by the pharmaceutical companies (Bailone et al., 2017).

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of the Farm

This study was done in early January 2018. There were five buffalo farms involved in this study and were selected randomly. Three farms located at Kulim district and another two located at Kuala Ketil and Pendang district, respectively. The farms were assigned name according to alphabetical order of A, B, C, D and E respectively based on the order of the sample being taken. All the farm was using the integrated semi intensive farming system, in which the animal was released for grazing in the oil palm estate, except for Farm E, as their animals were released in the grazing paddock. All the farm has Murrah breed of *Bubalus bubalis* as their breed of choice for milking animals. All the farm except for Farm E was using hand milking due to low number of milking animal. Farm E was using portable milking machine. Except for Farm E that are using Artificial Insemination, the rest of the farm were practicing natural mating as the means of getting their animal pregnant.

3.2 Milk Sampling

The milk was collected directly from the bulk tank of each farm. The milks were thoroughly stirred before collection. At least 50 ml of unpasteurized milks were put inside the whirl pak. Two whirl pak are needed for each farm, as the sample will be divided for Pusat Pembangunan Industri Tenusu (PPIT) and Makmal Veterinar

Kawasan Bukit Tengah (MVKBT). After that, the whirl pak was sealed and put inside a plastic bag. The plastic beg was tied and put into the polystyrene ice box with dried ice.

3.3 Sample Transportation

The milk samples were placed inside the polystyrene box with dried ice. The temperature of the polystyrene box was maintained and monitored at temperature of <4 ºC. The samples were transported to the PPIT and MVKBT at the same day as the collection.

3.4 Sample Processing

There are several parameters being used in this study to evaluate the quality of buffalo milk which were the compositional, physical-chemical characteristic and also hygienic and sanitary characteristic. In order to determine this parameter, five tests were indicated such as the Alcohol test, Solid Non-Fat (SNF), Methylene Blue Reduction test (MBRT), Antibiotic residue test, Total Plate Count (TPC) and Total Solid (TS). The first four parameters were determined at the nearest PPIT to the farm location, which is the PPIT Kulim for farm A, B and C and while for far, D and E, the first four test were conducted at PPIT Sungai Petani. For the determination of TDS and TPC, it was done at MVKBT.

3.4.1 Alcohol Test

2 ml of unpasteurized milk was pipetted from the whirl pak and put inside a test tube filled with 2 ml 80% alcohol (ration of 1:1). The contents of the test tube were mixed thoroughly and examined for any coagulation. If coagulation has occurred, fine particles of curd will be visible on the inside surface, presence of flake or curd denotes positive alcohol test.

3.4.2 Methylene Blue Reduction Test (MBRT)

9 ml of unpasteurized milk was pipetted from the whirl pak into a test tube. After that, 1 ml of methylene blue was pipetted into the test tube making the total volume inside the test tube 10 ml. The test tube was then incubated inside an incubator at temperature of 37.8 ºC for four hours. The test tube was checked every half an hour (30 minutes) for any changes in color of the test tube content from blue to white. If there's no color changes observed after four hours, the test was considered negative.

3.4.3 Antibiotic Residue Test (Delvotest® SP NT)

The ampoule containing Delvotest® reagent was detached from the plate in block based on the number of milk sample to be analyzed. The aluminum foil that covering the ampoule was perforated carefully as not to contaminate the reagent content inside. Milk sample from the whirl pak was well stirred before 0.1 ml of milk sample was pipetted from the whirl pak into the Delvotest® ampoule. The pipette was cleaned with running tap water for different milk sample from each farm. After that, the

ampoules + milk sample was incubated in the Delvotest® incubator for 3 hours at temperature of 64 ºC. After 3 hours, the ampoule was checked for any changes in color. Originally, the ampule reagent was purple in color. If there any antibiotic residue inside the milk sample, the ampoule reagent color will stay purple in color, indicating positive result, while if there was no antibiotic residue inside the milk sample, the ampule reagent purple color will turn yellowish, indicating negative result.

3.4.4 Total Solid (TS), Solid Non-Fat (SNF), Lactose, Fat & Protein Content

Determination of TS in the buffalo milk was done at PPIT Sungai Petani by using automated machine, Lactoscan Milk Analyzer. The machine was first set up to analyze buffalo milk. After that, approximately 5 ml of unpasteurized milk was poured into the container. The container then was put under suction panel of the machine. As the machine suck up the milk for analysis, the results will come out after 50 seconds. The results are printed out as shown in Appendix 1.

3.4.5 Total Plate Count (TPC)

Serial dilution of 10^{-1} until and $10^{-5}$was done by adding 1 ml of milk from the whirl pak into 9 mml of diluent. 1 ml of sample from the dilution tube 10^{-3}, 10^{-4} and 10^{-5} was added onto the center of the Petrifilm. The Petrifilm was incubated at 32 ºC for 48 hours. The colonies that grow on the Petrifilm was counted using standard colony counter and was recorded.

CHAPTER 4

RESULTS

4.1 Alcohol Test, Antibiotic Residue Test (Delvotest® SP NT) and Methylene Blue Reduction Test (MBRT)

The results for alcohol test of each farm are shown in Table 2. All the farms were negative for alcohol test, Delvotest® SP NT and MBRT.

Table 2: Results of alcohol test, Delvo test and MBRT of milk from each farm.

Farm	Alcohol test	Antibiotic residue test (Delvotest® SP NT)	Methylene Blue Reduction Test (MBRT)
A	negative	negative	negative
B	negative	negative	negative
C	negative	negative	negative
D	negative	negative	negative
E	negative	negative	negative

4.2 Total Solid (TS), Solid Non-Fat (SNF), Lactose, Fat & Protein Content

The results for Total Solid (TS), Solid Non-Fat (SNF), Lactose, Fat & Protein content of each farm are shown in Table 3. Farm C have the highest value of the composition measured which are, total solids (17.97 %), solid non-fat (9.34 %), fat (8.6%), protein (4.26%) and lactose (4.17 %) respectively. Farm A have the lowest value of total solids and fat which are 14.12% and 6.26 % respectively, while for solid non-fat, protein and lactose, farm B have the lowest value of 6.36 %, 2.86 % and 2.83 % respectively. The average value of each composition was calculated; total solids (15.06 %), solid son-fat (7.83 %), fat (7.22 %), protein (3.56 %) and lactose (3.48 %).

Table 3: Total Solid (TS), Solid Non-Fat (SNF), Lactose, Fat & Protein content of milk from each farm.

Farm	Total Solids (%)	Solid Non-Fat (%)	Fat (%)	Protein (%)	Lactose (%)
A	14.12	7.86	6.26	3.58	3.48
B	14.14	6.36	7.78	2.86	2.83
C	17.97	9.34	8.6	4.26	4.17
D	14.6	8.14	6.46	3.71	3.61
E	14.45	7.47	6.98	3.39	3.32
Average	**15.06**	**7.83**	**7.22**	**3.56**	**3.48**

4.3 Total Plate Count (TPC)

The results for TPC of each farm are shown in Table 4. TPC results demonstrated similar total count of bacteria per ml of colony forming unit (cfu) in each farm which was $<1.0x10^5$. All farm showing the colony forming unit (cfu) per ml of sample less than $1.0x10^5$.

Table 4: TPC results of milk from each farm.

Farm	A	B	C	D	E
Results (cfu/ml)	$<1.0x10^5$	$<1.0x10^5$	$<1.0x10^5$	$<1.0x10^5$	$<1.0x10^5$

CHAPTER 5

DISCUSSION

The composition of buffalo milk has been studied and reviewed by many researchers throughout the years (Ganguli, 1974). In general, buffalo milk contains higher proportion of all major constituents than cow milk (Park et al., 2008). Even though most of buffalo milk compositional studies are on the milk from Murrah breed, milk from most buffalo are almost similar with only slight difference (Sharma et al., 1982). Thus, in this study, the standard value of buffalo milk composition was used as stated in the Handbook of Milk of Non-Bovine Mammals (2008.) that is on Murrah breed. Pasquini et al. (2017) stated that, the combined effect of environment and genetics on the final quality of milk can be determined by evaluation of bulk buffalo milk composition. According to Abd El Salam and El- Shibini (2011), different factors such as breed, age, parity, stage of lactation, seasonality, feeding, udder disorders such as mastitis, and genetic polymorphism of milk proteins will affect the composition of buffalo milk. Total Plate Count are commonly used as indicators to evaluate the hygiene of the entire production process (Pasquini et al., 2017).

The present study shows that all farm was negative for Alcohol test, Antibiotic Residue test (Delvotest® SP NT) and Methylene Blue Reduction test (MBRT). This comply with the standard set by Malaysia Food Regulations (1985). Even though the standard was set for the use of cow milk, but taking into account of lack of guidelines for buffalo milk in Malaysia, it is being use as a standard for buffalo milk in this study. This is because, by definition in Malaysia Food Regulations (1985), milk, raw milk or

fresh milk is defined as 'the normal, clean, fresh mammary secretion of healthy cow, buffalo, goat or sheep that is properly fed and kept, excluding that obtained during the four days immediately following calving. There is also should be no trace of antibiotic substances present in the raw milk and when subjected to the Methylene Blue Reduction test (MBRT), it shall not completely decolorize the methylene blue solution in less than 4 hours (Malaysia Food Regulations, 1985). The principle of Delvo SP NT test is based on the diffusion of inhibitory substances (Antibiotic) present in the milk sample into agar. When there is presence of antibiotic, it will reduce growth and acid production by the test organism, and delays or prevents the agar from changing color from purple to yellow, thus making the test result as positive (Stead et al., 2008). As stated in Handbook of Milk of Non-Bovine Mammals (2008), the standardized value for buffalo milk fat content is 7.0%, Protein is 4.0%, Lactose is 5.1%, Solid Non-Fat (SNF) is 9.8% and Total Solid (TS) is 16.7%.

The present study shows the average percentage of total solids of buffalo milk to be 15.06%, which is lower than the standard value of 16.7%. Higher total solids content than the present study also being reported by Han et al. (2007) in which 16.69% for Murrah breed, while 17.14% for Nili-Ravi breed. As studied by Khedkar et al. (2016), the total solids of buffalo milk from buffalo of Italy, Egypt, USSR and India are 16.86 %, 16.40 %, 18.00 % and 17.02 % respectively, thus much higher than the present study. Wanapat & Chanthakhoun (2015) stated in their study that the total solid of buffalo milk is 16.30% which in turns higher than the present study. However, Kanwal et al (2004) finds the average buffalo milk total solids in their study to be slightly lower than the present study, which is 14.04%. Macedo et al. (2001) found a much higher average level of total solids in buffalo milk, which is 17.01% while Bailone et

al. (2017) is 15.89%. Another study done by Hashmi and Saleem (2014) found the average total solids to be 16.38%. In addition, Bailone et al. (2017) able to find a significant relationship between percentages of total solids with seasonal changes.

The present study shows the average SNF value of 7.83%, which is much lower compared to standardized value of 9.8%. Higher value of SNF also shown by study done by Khedkar et al. (2016), as the SNF of buffalo milk from buffalo of Italy, Egypt, USSR and India are 9.64%, 10.03%, 10.00% and 9.96%, respectively. Kanwal et al. (2004) stated that the average SNF content of buffalo milk to be 8.79%, which is slightly higher than the present finding. Pasquini et al. (2017) stated that in their finding of average SNF to be higher than the present study, which is 9.86%.

The average fat content reported in the present study (7.216%) was slightly higher than that the standard value. Present study also shown that the average buffalo milk fat content (7.216%) was slightly higher than that reported by Pasquini et al. (2017), which is 7.13%. This is also higher compared to what has been reported by Zotos and Bampidis (2014) in Greek buffalo milk. The monitored fat values were also lower than those reported by Rosati and Van Vleck (2002) for individual milk samples produced by buffaloes reared mainly in the south of Italy (average value 8.59 ± 0.85%). Additionally, Di Francia et al. (2007) found higher fat content in milk produced by Italian buffaloes fed a ration containing extruded peas or soybean cake as concentrate components (7.84 and 7.56%, respectively). Finally, the fat values observed in the present study were slightly higher than the values reported by Cunha Neto et al. (2005) for Brazilian buffalo milk used for the production of yogurt and by Enb et al. (2009) for milk produced by Egyptian buffaloes and used for cheese manufacturing. Khedkar

et al. (2016) reported that the average fat content for buffalo milk from Italy, Egypt, USSR and India are 7.22%, 6.37%, 8.00% and 7.06%, respectively. The buffalo milk from Italy and USSR are higher as compared to the present study, while the buffalo milk from Egypt and India are lower as compared to the present study.

Khedkar et al. (2016) mentioned that, the protein that contains in the buffalo milk are of high quality because they contain all the essential amino acids required by the human body. In addition, 80% of the total protein contained in buffalo milk are being casein and all casein of buffalo milk is present in micellar form (Park, 2008). The average protein content reported in this study (3.56%) was lower as compared to the standard value. Findings from Asker et al. (1957), Ganguli (1974), Ragab et al. (1958) and Sarswat (1985) shows that, the average protein content of the present study also lower than what they have reported (3.8-4.3%). The protein content of buffalo milk from buffalo of Italy, Egypt, USSR and India are 3.95%, 3.87%, 4.32% and 3.90%, respectively (Khedkar et al., 2016). This is much higher compared to our findings. Kanwal et al. (2004) stated that the average protein content of buffalo milk to be 3.87%, which is slightly higher than our finding. Han et al. (2007) reported the buffalo milk protein content to be 4.27% for Murrah breed, while 4.16% for Nili-Ravi breed. This is also much higher than what we found in our study.

High lactose content in buffalo milk can be a good source of energy for many body activities especially for the functional aspect of the brain and regulation of hormones in the body (Bailone, 2017). The average lactose content of buffalo milk in this study is 3.48% which is much lower than the standard value of 5.1%. Bailone et al. (2017), Wanapat & Chanthakhoun (2015), Kanwal et al. (2004) and Pasuquini et al. (2017)

reported the average lactose content to be 4.87%, 5.00%, 3.92% and 4.77%, respectively. All of this are much higher compared to the present study. Higher lactose content than the present study also being reported by Han et al. (2007) in which 5.07% for Murrah breed, while 4.56% for Nili-Ravi breed. As studied by Khedkar et al. (2016), the lactose content of buffalo milk from buffalo of Italy, Egypt, USSR and India are 4.88%, 5.00%, 4.96% and 5.28% respectively, thus much higher than the present study.

The present study shows that all of the farm having TPC count less than $1.0x10^5$ cfu/ml. This comply with the limit set by Department of Veterinary Services, Malaysia which is not to exceed the count of 10^6 cfu/ml. The reasons for such good TPC counts could be due to healthy udder of cow, good hygienic milking procedures or equipment, as well as a proper milk storage conditions (Chye, 2004). Furthermore, the milk sample for TPC collected from each farm is freshly taken after milking and subjected to TPC procedure on the same day of sample collection. According to Aumaitre (1999), the health of the dairy herd, milking and pre-storage conditions are also basic determinants of milk quality. Bacteria may enter milk while it is in the udder and most of the organisms in raw milk are contaminants from the external surface of udder, milking utensils and handlers (Ayres et al., 1980). Various types of equipment and utensils, such as milking machines, pails, cans and milk churns are used in handling milk on the farm. In order to reduce contamination of milk, utensils used for milking should be rinsed, cleaned using detergent and disinfected immediately after use (Dodd and Phipps, 1994; FAO and WHO, 1997). The use of detergents and good quality water for cleaning the equipment could be expected to remove milk remains including micro-organisms and thereby affect the microbiological quality of milk

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In summary, the composition of buffalo milk such as fat, protein and lactose, produced in the state of Kedah are slightly lower than the standard value stated in the Handbook of Milk of Non-Bovine Mammals and published journal and article from previous findings. Having said so, the buffalo milk produced in Kedah able to meet other quality parameters set in this study such as keeping quality, stability, microbial load and antimicrobial residue. Therefore, it is concluded that the buffalo milk that being produced in the state of Kedah is of good quality.

For further study in the future, it is recommended, that the number of sample collected to be increased so that the results obtained will be of higher reliability. It can be achieved either by focusing the study on one particular farm and accessing the milk quality being produced by individual animals or by increasing the number of farm. By doing this, not only that our sample size will be bigger, but we can also see the relationship between several parameters being described in published article that affect the outcome of the milk quality such as feed, udder health, breed, and etc.

Periodically analysis of buffalo milk can also be done in the future in which the sample are collected over a period of monthly, yearly or even daily. By doing this, statistical analysis can be done in order to determine the significant effect of seasonal changes over the milk quality parameter such as composition, physical-chemical characteristic and others. Lastly, we would like to recommend for a longer course of study in order

to be able to efficiently get a bigger sample as the farm location are far from each other.

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APPENDIX 1: Pictures