UNIVERSITI PUTRA MALAYSIA

EVALUATION OF QUALITY AND COMPOSITION OF DAIRY BUFFALO MILK IN SELANGOR, MALAYSIA

NOR ASRINA BT MOHAMMAD MUKHELAS

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EVALUATION OF QUALITY AND COMPOSITION OF DAIRY BUFFALO MILK IN SELANGOR, MALAYSIA

NOR ASRINA BT MOHAMMAD MUKHELAS

A project paper submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia in partial fulfilment of the requirement for the

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Evaluation of Quality and Composition of Dairy Buffalo Milk in Selangor, Malaysia" by Nor Asrina Bt Mohammad Mukhelas 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 - Final Year Project.

PROF. DR MD ZUKI BIN ABU BAKAR@ZAKARIA
DVM (UPM), PhD (GLASGOW)
Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

ASSOC. PROF. DR HASLIZA BINTI ABU HASSIM
D.V.M. (UPM), MVSc (UPM), PhD (Ghent University, Belgium)
Department of Veterinary Preclinical Sciences
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-supervisor)

DEDICATIONS

This final year project is dedicated to:

The most Merciful and Almighty God, my creator. Allah S.W.T,

My beloved family,

Abah, Mohammad Mukhelas B Hj. Naruwi

Mak, Kamariah Bt Hassan

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Kakak, Nor Fadilah

Safwan Affendi,

My best friends,

My classmates DVM 23,

And for the animal kingdom

Thank you for not giving up on me to finish this degree, and giving me unconditional physical and mental support.

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

PENILAIAN KUALITI DAN KOMPOSISI NUTRIEN SUSU KERBAU DI SELANGOR, MALAYSIA

Oleh

Nor Asrina Bt Mohammad Mukhelas

2022

Penyelia: Professor Dr Md Zuki Abu Bakar

Penyelia bersama: Assoc. Prof. Dr Hasliza Abu Hassim

Kerbau mempunyai daya ketahanan yang tinggi terhadap iklim tropikal dan kemampuan untuk memanfaatkan serat yang berkualiti rendah. Oleh itu, susu kerbau mempunyai potensi untuk dipasarkan dalam skala yang besar sama seperti susu lembu. Namun begitu, susu kerbau tidak dikaji secara meluas di Malaysia berbanding susu lembu. Jadi, objektif kajian ini adalah untuk menentukan kualiti dan komposisi nutrien susu kerbau dihasilkan oleh peladang kecil tempatan. Sebanyak empat sampel susu telah dikumpulkan daripada empat ladang tempatan terpilih di Selangor, Malaysia. Kualiti susu kemudian dinilai melalui beberapa ujian iaitu Gravity Tertentu, Beku Semasa Mendidih, Ujian Alkohol, Ujian Resazurin, Ujian Penurunan Warna Metilena (MBRT), Metodologi Hitungan Cawan, dan Hitungan Plat Kolifom. Manakala, penilaian untuk komposisi nutrien, susu dinilai mengunakan Milkoscan FT2. Kajian ini mendedahkan, berdasarkan parameter yang dikaji, sebahagian besar dari susu kerbau mempunyai kualiti yang tinggi. Manakala untuk komposisi nutrien beberapa susu menunjukkan nilai yang tinggi seperti protein, pepejal tanpa lemak, dan urea. Sementara nutrien yang lain seperti lemak, laktosa, jumlah pepejal dan acid sitrik menunjukkan nilai yang rendah daripada nilai piawai yang dilaporkan untuk susu kerbau. Umumnya susu kerbau mempunyai kualiti yang tinggi dan mempunyai komposisi nutrien yang bagus. Oleh itu, susu kerbau mempunyai kemungkinan yang tinggi untuk dikomersialkan di Malaysia. Ladang kerbau tenusu perlu diselia dengan baik di Malaysia dengan menambah baik sistem pengurusan bagi menghasilkan susu yang lebih berkualiti.

Kata kunci: susu; kualitu; komposisi nutrien; peladang kecil

ABSTRACT

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

EVALUATION OF QUALITY AND COMPOSITION OF DAIRY BUFFALO MILK IN SELANGOR, MALAYSIA

by

Nor Asrina Bt Mohammad Mukhelas

2022

Supervisor: Professor Dr Md Zuki Abu Bakar

Co-Supervisor: Assoc. Prof. Dr Hasliza Abu Hassim

Asian buffalo (*Bubalus bubalis*) is most likely domesticated for its strong and muscular features which are fitted for sources of draft power, transportation, meat and milk. Milk consists of numerous nutrients that help growth development and support many bodies' vital functions. It is important to consume high-quality milk to ensure the body meets most of the nutrient requirements. Due to buffalo's high adaptability to tropical climates and capability of utilizing low-quality roughages, especially agricultural crop residue and by-products in developing countries, there is potential that buffalo milk can be commercialized on a larger scale as dairy cow milk. However, buffalo milk has not been widely studied in Malaysia compared to dairy cow milk. Hence, the objective of this study is to determine the quality and nutrient composition of buffalo milk produced locally by smallholders. A total of four buffalo milk samples were collected from four selected farms in Selangor, Malaysia. The quality of milk samples was then evaluated through a few tests which are specific gravity, clot on boiling, alcohol test, Resazurin test, Methylene Blue Reduction test (MBRT), Total Plate Count, and coliform plate count. While for the evaluation of milk composition, milk was analyzed by using Milkoscan™ FT2. This study revealed that, based on the milk quality parameters evaluated, most buffalo milk possesses high-quality milk. While for the nutrient composition, some of the nutrients showed higher values such as protein, solid non-fat, and urea. While others, such as fat, lactose, total solid and citric acid showed lower than the standard value reported for buffalo milk. Generally, buffalo milk has high milk quality and good nutrient composition. Thus, there is a high possibility that buffalo milk can be commercialised. Dairy buffalo farms should be well-established in Malaysia to improve their management system and produce more quality milk.

Keywords: buffalo milk; quality; nutrient composition; smallholders

CHAPTER 1

INTRODUCTION

Milk by definition is a fluid secreted by the mammary glands of females for the nourishment of their young (Merriam Webster, 2022). But nowadays, milk is not just for young but instead all stages of age can drink a cup of milk and it became more popular since. Milk can bring many advantages for human being. It can improve bone health by increasing bone density and reduce risk of osteoporosis (Rizzoli, 2022) other than that, Guoyao (2016) found that milk help to reduce age-related muscle loss and induce muscle repair after exercise. Besides, milk also packed with number of nutrients to support vital functions of our body. This include promote growth development, immune regulation, and cellular repair. Although dairy milk can be good to certain people, but some of them cannot digest lactose which may lead to diarrhoea and bloating. Technology has led to various types of non-dairy milk for those who are lactose intolerant which are soy milk, oat milk, almond milk and other plant-based milk.

The domestic water buffaloes are large animals belong to the family Bovidae, which are among crucial livestock species most of south America, southern Asia, northern Africa, and southern Europe. There are approximately 158 million water buffalo in the world. They are particularly suited to the marshland conditions in East and Southeast Asia (Hoffman & Cawthorn, 2014). Water buffalo or scientifically known as *Bubalus bubalis* can be further categorised into two subspecies, river buffalo and swamp buffalo (Minervino et al., 2020). Purpose of these two subspecies are different as swamp buffalo is reared for draft and meat while river buffalo was for milk and also for meat.

Buffalo milk production accounted for about 15.14% of global dairy milk production in 2018 according to Food and Agriculture Organization (FAO) while another 80% are from cattle milk. As for in Asia, major buffalo milk productions are from India and Pakistan which contribute 35.30% from the total milk production (Minervino et al., 2020). Malaysia's buffalo milk production is varied from 4.00 to 8.00 L/day. While, local Murrah crossbred buffalo can produce an average of 4.7 L milk/day/animal/lactation period which contribute about a total of 1000 L milk/animal/year which is far from meeting local milk demand (Azmi et al., 2021).

To be able to evaluate the quality of buffalo milk, usually it is divided into physical properties, evaluation of quality, and nutrient composition. Physical properties of milk that have been studied and analysed between species include viscosity, specific gravity, and freezing point. Milk contains more water than any other element, with the rest of the composition being total solids (TS). Total solid includes fats, proteins, sugars, vitamins, and minerals. When fat is excluded from the TS component, the rest is known as solids non-fat (SNF). Other than that, FAO suggests that there are a few aspects of milk that can be tested for which are quantity, organoleptic characteristics, compositional characteristics, physical and chemical characteristics, hygienic level, drug residue and adulteration.

The milk quality evaluation in this study only focuses on physical characteristics, chemical composition, and the contamination level of the milk samples. To evaluate the heat stability of the milk, Clot on Boiling and Alcohol Test need to be carried out. Resazurin Test, Methylene Blue Reduction test (MBRT), Total Plate Count test are to study the level of overall microbial activity in the milk sample. While Coliform Plate Count Test is to determine any indication of fecal contamination. Lastly to determine the composition of the milk is will be analysed with Milkoscan™ FT2.

1.2 Justification

Nutrient composition and milk quality of dairy buffalo milk has not been widely studied as compared to dairy cow milk. It is believed that buffalo milk has advantages over the cow's milk and its production can be established and commercialised in our country. Hence, this study could help to generate data on the quality of buffalo milk in Selangor area since there is a paucity of information on buffalo milk in Malaysia and lack of studies reported regarding this matter. Besides, the outcome of this study can help farmers to improve their management of rearing dairy buffalos to increase their milk quality ultimately could reduce the restriction of full potential of buffalo milk to be commercialised and produced on a larger scale. Therefore, the main objectives to achieve in this study is to evaluate the quality of buffalo milk and to determine the nutrient composition of buffalo milk.

1.3 Hypothesis

Ho Locally produced dairy buffalo milk by smallholders in Selangor has low quality and low nutrient composition.

Ha Locally produced dairy buffalo milk by smallholders in Selangor has good quality and high nutrient composition.

1.4 Objective

This study was conducted to evaluate and determine the quality and nutrient composition of locally produced dairy buffalo milk by smallholders in selected farms in Selangor.

CHAPTER 2

LITERATURE REVIEW

2.1 Milk

Milk is a major source of dietary energy, protein, and fat. According to Khedkar *et al.* (2016), It also could be defined as almost complete food in human diet as it contains all essential nutrients that help to nourish and promote the growth of young mammals. Milk can be the most versatile of all animal-based commodities, because milk turns into any physical form such as cheese, yogurt, ice cream, ghee, milk powder and other form of liquid. Development of technology in the past few decades have significantly affect the variety of dairy product available in the market during this present time (Muehlhoff *et al*., 2013.)

Traditional western diets, especially in cold climate in United States (US), had include the dairy milk products as part of the diets. The recommended intake of milk in the US is 237ml serving per day for children 9 years of age and above and for adults (Haug *et al*., 2020). In East and Southeast Asia, about 24.9 kg/year per capita of milk is consumed, contributing on average 134 kcal of energy/person/day, 8g of protein/person/day and 7.3g of fat/person/day. Milk consumption is increasing faster than meat consumption (Muehlhoff *et al*., 2013.) Shahbandeh (2022) discover that India is the largest country that consumes milk which drinks about 83 million metric tonnes in 2021. The next country is followed by European Union which is about 23.9 million metric tonnes in the same year. While in Malaysia, Suntharalingam (2019) reports that milk consumption increased fourfold between 2011 to 2017 which is 18.9 million litres and 62.80 million litres in the respective year. This increase in demand for milk may be due to rising income, change in taste of preference and increase awareness of the nutritional benefit of milk

2.2 Buffalo

According to Intergrated Taxonomic Infromation System Report, taxonomically, buffalo can fall under order of Artiodactyla characterised as even-toed ungulates or hoofed animals in which bear weight equally on two of their five toes: the third and fourth (Owen, 1848). Other animals that fall under this order are pigs, deer, giraffes, antelope, sheep, goat etc. Further down in taxonomy buffalo can be classified to family of Bovidae, subfamily Bovinae, genus Bubalus.

Study from Minervino *et al.* (2020) found that *Bubalus*, was widely distributed in Asia and Europe during Pleistocene. Wild Asian buffalo, *Bubalus arnee* that is native to northern India and inhabits in marshes and the jungle, is one of the species of wild animals in this genus. This large animal can be gray-black, dark grey, or dark brown, and it can weigh up to 1,000 kg. It has huge horns that are separate from the base, and it can reach heights of up to 200 cm. The most accepted theory is that wild Asian buffalo is the most likely ancestor of water buffaloes as it supports two independent domestication events from river and swamp buffaloes.

The river buffalo (*B. bubalis bubalis*) and the swamp buffalo are the two subspecies of the water buffalo. Molecular studies reveal that swamp buffaloes exhibit a remarkable diversity of maternal and paternal lineages, they also exhibit strong phenotypic homogeneity and genetic differentiation that is influenced by geography, and lack of gene flow. Swamp buffaloes typically weigh between 325 and 450 kg and are smaller and lighter than river buffaloes. They are primarily raised for draft purposes, but also could produce large quantities of milk (up to 600 kg of milk per year). Swamp buffaloes are predominant in Southeast Asia and Australia. There are many types of breeds of water buffalo under swamp buffalo subspecies. For example, Carabao breed, the colour of their body coat ranging from light grey to slate grey. Their horns are either formed like a sickle or bent backward toward the neck. They are bred for work, are good for making meat, and have white spots on their legs. Other than that, Chinese buffalo is also a swamp-type buffalo that used mostly for draft work. They are very efficient in marshland, particularly in rice field. Both of these breeds are really poor in milk production capacity.

In other hand, River buffaloes are typically larger than swamp buffaloes. It can reach weight between 450 and 1,000 kg. The majority of breeds have curled horns and are primarily found in India, Pakistan, as well as several European, western Asian, and also American countries. According to Minervino *et al*. (2020), There are about three international river breeds are widely distributed all around the word, due to their high genetic potential for milk production. The breed includes Italian Mediterranean, Nili-Ravi and Murrah. Murrah is the most well-known dairy buffalo breed in the world. In northwest India, it is chosen for milk production and is renowned for its curled horns, huge stocky animals, and heavy bones. The second most represented breed in the world is Nili-Ravi. The breed resembles the Murrah breed but differs despite having walled eyes, white patterns on its extremities, and less curled horns. Although most members of the breed have black skin, there are few who are albino, brown, spotted, or have clear eyes. The most significant breed of dairy buffalo in Asia, chosen for this purpose, in which the breed could produces 3,050 kg of milk annually. Apart from that, Italian Mediterranean is exclusively black, differ from European Mediterranean which they have black, dark gray and brown coats. It is a compact breed with deep and wide chest but have short back and rump. The daily milk production average varies greatly and is influenced by things like genetics and the feeding regime. For animals that are poorly fed, it might range from 3 to 4 kg milk per day to 15 kg milk per day in intensive production systems. the Italian Mediterranean breed has a high milk yield and quality. Since that it was chosen to create a dairy breed tailored specifically for the cheese industry.

In Malaysia, a study by Azmi *et al*. (2021) discover that Murrah crossbred buffalo can outperform swamp buffalo in terms of biological performance in which Murrah crossbred have higher pre- and post-weaning daily weight gain thus taking shorter period to achieve market and breeding weight. As a result, raising Murrah crossbred buffaloes with improved intervention management practices would enhance a farmer's net profit.

In addition, Minervino *et al*. (2020) found that Buffalo can be affected by the same parasites and diseases as cattle, but varying degrees of tick resistance between the buffalo breed. However according to FAO (2000) water buffalo are less vulnerable to parasite and tick infestation due to their behaviour of wallowing in muds to keep their body cool. Buffalo has a great capacity for adaptation and survival as well as a higher capacity to metabolize feed with poor nutritional content. They play a crucial role in the economy of many tropical and subtropical countries as they are highly adapted to hot and wet floodplains (Minervino, 2020)

2.2 Buffalo Milk

Buffaloes are the second largest milk producers in the world, however, there is still a huge gap between buffalo milk and cow milk produced annually (Emond, 2019). Food And Agriculture Organization (2022) claims that most of the world's dairy milk production comes from cattle which contribute about 81% followed by buffaloes 15%, and 3% from goats and sheep while the remaining is produced by camels, equine and yaks. According to Minervino (2020), about 97.59% of the world's production of buffalo milk is produced by the top 10 nations which are India, Pakistan, China, Egypt, Nepal, Italy, Myanmar, Iran, Colombia, and Brazil. In contrast to cattle milk, which climbed by 10.67% (from 617 to 683 million tons), the global production of buffalo milk increased by 32.57% between 2011 and 2018 (from 96 to 127 million tonnes). Furthermore, buffaloes are the main milk-producing animals in India and Pakistan, producing 35.30% of all the produce milk produced in Asia.

On the other hand, in Europe, Italy, which ranks fifth in buffalo milk production worldwide contribute about 97% of the buffalo milk in Europe (Minervino, 2020). Italy, Turkey, Bulgaria, and Greece are among the top buffalo milk producers in Europe despite Greece being the former is the world's eighteenth-largest buffalo milk producer (FAOSTAT, 2013). Cazacu *et al*. (2014) claim that the buffalo milk industry is considered as being relatively new since it has only recently started to develop. Mozzarella cheese produced from buffalo milk is a valuable delicacy, especially in Italy, US, Germany, France, Japan and UK (Han *et al*., 2012; Tripaldi, 2005; Borghese, 2005). On the other hand, buffalo milk is much preferred by consumers due to its high nutritional value and the ability of buffalo milk can be transformed into other valuable goods like curd, yoghurt, cheese and ice cream (Han *et al*., 2012). Moreover, Ice cream from buffalo milk could improve the texture of ice cream by making it smoother and more compact (Borghese, 2005).

2.3 Factors Influence Composition of Buffalo Milk

A study has discovered that the gross composition of buffalo milk is affected by several factors which are breed, lactation number, stage of lactation and seasonality, feeding and incidence of subclinical mastitis (Abd El-Salam *et al.*, 2011). While in another study, Minervino *et al*. (2020) reported that milk yield is affected by genetic background, health status, period of calving, season, and environmental factors such as climate conditions, feeding, and welfare. Genetic potential is the most important factor, as represented in the Italian Mediterranean, Nili-Ravi, and Murrah breeds. The differences in concentration of protein, fat, lactose, ash, and consequently dry matter, which were found to be higher in buffalo milk than in cow milk, could be believed to be due to the physiological differences of the animal, stage of lactation, and some common factors like season, feed, breed, time, and sequence of milking (Ahmad *et al*., 2008).

In addition, buffalo milk and its derived products could be a good source of conjugated linoleic acid (CLA). CLA has been linked to a wide range of physiological benefits, including those connected to its potential antiadipogenic, antidiabetic, anticarcinogenic, and antiatherosclerotic properties (Belury, 2002). French *et al*. (2000) discover that conjugated linoleic acid (CLA) concentration content in milk increased linearly when the animals were pasture fed and decreased when the grass intake decreased. The type of nutritional supplement supplied to dairy animals can be changed to affect the CLA concentration of milk fat. For instance, supplementing the diet with polyunsaturated oils that contain either corn oil or sunflower oil could increase CLA content significantly. Another study by Khanal and Oslon (2004) also supports that the main factor influencing the concentration of CLA in foods like milk, meat, and eggs is the animals' diet.

2.4 Comparison of Buffalo and Cow Milk

Study by Minervo *et al*. (2020) found that buffalo milk is estimated to have twice as high in fat content about 30% higher in total solid compared to cow's milk. This is also supported by other studies that said buffalo milk contains higher amounts of protein, casein, fat, lactose, calcium, vitamins, and minerals compared to cow's milk

(Tripaldi, 2005; Han *et al*., 2012). The comparative composition of buffalo and cow milk is demonstrated in Table 1. Buffalo also had a greater value in terms of dry matter basis compared to cow's milk (Minervo *et al*., 2020). Buffalo milk had higher total nitrogen content, fat, and lactose contents than cow milk did, while normal pH values of the milk were the same from both species (Ahmad *et al*., 2008). While in term of energy, 1 kg of buffalo milk equals to 5.1 Mj which is twice much higher as 1 kg of cow's milk which equals to 2.90 Mj (Minervo *et al*., 2020).

Buffalo milk is ideal for processing various varieties of yoghurt because it has higher levels of total solids and casein, as well as nearly twice as much butterfat as cow milk. This results in creamy textures and rich flavour profiles for the yoghurt (Han *et al*., 2012). Probiotics including *Lactobacillus acidophilus (L. acidophilus), Lactobacillus casei (L. casei),* and *Bifidobacteria* probiotic bacteria are increasingly being used in various dairy products to being a mainstream if healthy food. Similar to how the content of cow and buffalo milk varies, changes in buffalo milk composition are attributed to breed, geographical location, and feeding; and these variations would strongly affect the manufacturing conditions, sensory quality, and nutritional properties of yoghurt products (Han *et al*., 2012).

According to Salaun, Mietton, and Gaucheron (2005), the acido-basic composition of milk affects its ability to act as a buffer. The difference between the milk of different species was likely caused by the increased casein concentration in buffalo milk compared to cow milk. Additionally, inorganic phosphate also contributes to the buffering capacity. Compared to cow milk, buffalo milk's ultracentrifuged pellet had a lower water content (1.90 g of water/g of dry pellet as compared to 2.24 g for cow milk). The structure and mineralization of casein micelles as well as casein's glycosylation are the most important variables that can influence these notable differences. The higher mineralization of casein micelles from buffalo milk was observed (Ahmad, 2008).

Water buffalo is more productive domestic animals to be compared to the cow as buffalo have longer longevity or productive life compared to cow (Minervo *et al.*, 2020). Compared to cattle, buffaloes are more productive and may produce calves and milk for up to 20 years of age (FAO, 2022). This could also prove the study by Peeva and Ilieva (2010) who reported that average longevity of buffaloes was 2636 days (about 7 years and 3 months). In comparison to another study by De Vries & Marcondes (2020) claims that longevity in cow is between 2.5 years to 4 years due to poor health, failure to conceive or conformation problems prior to culling. Longer longevity will improve the economics of dairy farms as the farmer need fewer replacement cows, less feed, and less manure and therefore reduced environmental footprint (Murawski, 2022).

Table 1: Average composition (%) of buffalo versus cow milk.

Milk constituent	Buffalo	Cow
Fat	7.0	4.3
Protein	4.0	3.4
Lactose	5.1	4.8
Mineral	0.8	0.7
SNF	9.8	9.0
Total Solid	16.7	13.3

Source: Handbook of Milk of Non-Bovine Mammals (2006)

2.4 Milk Quality Evaluation

Evaluation of milk quality prior to consumption is important to ensure food safety. Aside from being nutritious for consumption, milk also provides a favourable environment for the growth of microorganisms. Yeasts, moulds, and a broad spectrum of bacteria can grow in milk. Improper management during milking and handling can also lead to contamination. Dairy environments are often contaminated with common bacterial pathogens such as Salmonella, E. coli, and Campylobacter. These bacteria can cause diarrhoea, renal failure and in severe cases, death. Milk with higher levels of contamination also spoils very quickly due to microbial activity which causes the milk to become very acidic, producing sour flavours, then coagulated and finally cannot be used (Ebner *et al*., 2017). Moreover, the fact that milk contains high amount of nutrient content is also could be the best medium for microorganisms to rapidly replicate. However, contamination of mastitis milk into fresh clean milk may contribute to a high microbial load (Jeffery & Wilson, 1987).

Thus, this study is carried out to further characterizes the quality and physicochemical composition of dairy buffalo milk with respects to the physical properties and nutrient composition. Therefore, the analysis of Total Plate count (TPC), Methylene Blue Reduction Test (MBRT) and Resazurin Test can be good indicator for sanitary and hygienic status of the milk at the farm level. According to Fifteenth schedule of Malaysia Food Act 1983, the accepted limit of the bacterial colony count in raw milk is about 100,000 cfu/ml. anything above the limit considered contaminated. Poor milk quality is usually reported as one of the main causes for losses and results lower income for Malaysia's smallholder dairy farmers (Chye *et al*., 2004). Other than TPC, coliform test could also be done to determine the possible contamination by contaminated water, soil and manure. The bacteria such as *E. coli* and other coliform bacteria like *Enterobacter aerogenes, Citrobacter spp.* and *Klebsiella spp.* E. coli's presence raises the possibility that the sample also contains other enteric pathogens (Chye *et al*., 2004).

Besides, an analysis of milk also could be done to determine the parameter such as the amount of water, total solids, specific gravity, fat, protein and many other parameters. By doing so, the quality and physiochemical characteristics of the milk can be determined. The typical mammary secretion which results from fully milking a healthy dairy animal without either adding to it or taking anything away from it. It shall be free from colostrum. Milk of various sources and designations must adhere to the standard. Fats in milk are usually called as butterfat. Butterfat has less density than water making it rise and separate. Homogenization is a method of reducing the average size of the fat globule thus the fat will homogeneously mix for a longer period of time (Mohammed Abdelreda & Naji Ajmi, 2016)

CHAPTER 3

MATERIAL AND METHODS

3.1 Description of the Farm

This study was done in early September 2022, the buffalo farms that involved in this study was focusing mainly in Selangor area. There were four Buffalo farms selected for this study by using convenience sampling method. Two of the farms were from Ladang Angkat Programme. The farms were represented according to numbering. For example, 1, 2, 3, 4 respectively. It is based on the order of sample being taken from selected farms. All of the farms were using integrated semi-intensive farming systems, in which the buffalo was released for grazing in open pastures or in nearby oil palm plantations. In addition, all four farms use the same breed of dairy buffalo which is Murrah breed as their choice of milking animals. For feeding management, most of the farms provide Napier, pallets, salt block, and soy-based feed to their buffalo.

3.2 Milk Sampling

Milk was collected from all four buffalo farms. All farmers claimed that they do the milking on the same day as the samples were collected. Collection of raw milk samples was obtained from bulk collecting tank. About 1 litter of buffalo milk was collected for each of the farms and was placed into sterile plastic bag or plastic bottles.

3.3 Milk Sample Transportation and Storage

Ice box and dried ice was used to store the milk samples temporarily during travelling from one farm to another and back to the laboratory. The milk samples were stored and maintained at chilled temperature which is between 0℃ to 4℃ during transportation.

3.4 Evaluation of Milk Sample

To evaluate the wholesome and the quality of the milk sample there are a few parameters that need to be evaluated. The physical characteristics, chemical composition, and contamination level of the milk samples. Hence, there are 8 tests that need to be done to evaluate these parameters which are Specific Gravity, Clot on Boiling, Alcohol Test, Resazurin Test, Methylene Blue Reduction Test (MBRT), Total Plate Count Test, Coliform Test, and Milk Composition.

About 200 ml of buffalo milk samples from each farm were then sent to Pusat Perkhidmatan Industri Tenusu, Jabatan Perkhidmatan Veterinar Alor Gajah, Melaka (PPIT, JPV Alor Gajah, Melaka), Public Health Laboratory, Faculty of Veterinary Medicine, and Dairy Lab, Department of Animal Science, Faculty of Agriculture, UPM for milk evaluation. The milk samples were sent to PPIT, Alor Gajah, Melaka for Total Plate Count (TPC) and milk composition evaluation. The milk samples were evaluated for another 3 tests which are Alcohol Test, Methylene Blue Reduction test (MBRT), and Coliform Test in Public Health Laboratory, Faculty of Veterinary Medicine, UPM. While, the specific gravity, Clot on Boiling, and Resazurin Test was done in Dairy Lab, Department of Animal Science, Faculty of Agriculture, UPM.

3.4.1 Specific Gravity Test

The milk sample was gently swirled and pour 100 ml of milk into measuring cylinder or any container deeper than the length of the lactometer. Let the lactometer sink into the measuring cylinder. Read and record the reading of the lactometer (g/mL). Normal dairy buffalo milk should have a density between 1.028 to 1.032 g/mL. The aim of this test is to detect any changes in the density of the milk resulting from the adulteration of water or solids content in the milk.

3.4.2 Clot on Boiling (COB) Test

This test is primarily to determine the suitability of milk for heat processing. A 5 ml milk sample was poured into the test tube. Then, insert the test tube containing milk sample in a boiling water bath for 5 minutes. After that, take out the test tube from water bath without shaking it. Any precipitation on the sides of test tube were observed and recorded. Positive observation of COB test is noted when there is presence of clot or flakes, thus, it indicates the milk sample is rejected.

3.4.3 Alcohol Test

This test almost similar to COB test. It is to determine the suitability of milk for heat processing. About 2 ml of milk sample is placed into test tube. Add 2 ml 70% alcohol into the same test tube, and mix gently. After 5 minutes, observe and record if there any precipitation on the side of test tube. If milk sample shows any flakes or precipitation, it is suggested the milk sample is positive for an alcohol test. Ultimately, rejected from heat processing.

3.4.4 Resazurin Test

This test is important to assess the bacteria content in raw milk. As the bacteria in the milk increase, the faster the oxygen is removed in the milk, thus the blue dye become more discoloured. Add one tablet of resazurin into 50 ml distilled water to prepare resazurin solution. Note that this solution must keep away from sunlight. Transfer 10 ml of each milk sample into the test tube. Add 1 ml of resazurin solution prepared earlier into each test tube, close the test tube using a stopper and mixed them gently. Label the test tubes according to the respective samples and place them in 37℃ water baths for 1 hour. After 1 hour, take out the test tube from water bath, observe and grade the colour of the milk samples (according to the standard milk grading system as shown in Table 2) and compare each sample to the sample of milk without resazurin solution (control). The grading of 3 and lower indicate the milk have fair to bad quality of milk

Table 2: Grading of milk from Resazurin disk.

Resazurin disk no.	Colour	Grade of milk
6	Blue	Excellent
5	Light blue	Very good
4	Purple	Good
3	Purple-pink	Fair
2	Light-pink	Poor
1	Pink	Bad
0	White	Very bad

Source: Nath and Aravindkumar (2021)

3.4.5 Methylene Blue Reduction Test (MBRT)

The objective of this test is to evaluate the quality and microbial load in the milk. Methylene blue form blue colour due to presence of the oxygen. It becomes discolour when the oxygen is depleted as the result of metabolic reactions in milk.

10 ml of each milk sample is poured into sterile test tubes. Then, add 1 ml of the methylene blue into each test tube and mix gently. The test tubes were then placed inside 37°C water bath for 6 hours. Any complete decolourization or formation of the blue rings (0.5mm) occur at the top surface of the milk samples were observed for colour changes at regular intervals for 30 minutes. The time taken for the

reduction of methylene blue for each sample were recorded. According to Food Regulations 1985, Reduction test shall not completely decolourize the methylene blue in less than five hours. if no colour changes are observed in five hours. The test is considered negative, and the milk quality is good.

3.4.6 Total Plate Count (TPC)

Total plate count test is to measure overall bacterial levels. Higher bacterial level can reduce the shelf life of the milk. Thus, resulting low quality of milk. Nine ml of diluent was placed into 5 individuals sterile labelled test tubes (-1, -2, -3, etc). Add 1 ml of milk sample to the test tube labelled -1 and mix thoroughly. Take 1 ml milk mixed with dilution from labelled -1 test tube and put into labelled -2 test tube, then mix thoroughly. Repeat the step for the remaining test tubes. Then, take 1 ml sample from each test tube labelled -3, -4, -5 and added into each center of Petrifilm. The Petrifilm is then incubate at 37℃ for 48 hours. After that, count and record the number of colonies by using automated colony counter model. The result was recorded at 4^{th} dilution. According to Fifteenth Schedule (Regulation 39) in Malaysia Food Act 1983, TPC should be less than 1.0 x 10^5 cfu/ml to be considered as high-quality of milk.

3.4.7 Coliform Test

Coliform bacteria are usually found in the intestinal system of animals. Coliform test used as indicator to evaluate fecal contamination level of the milk sample. The method is the same as TPC but the agar used is Violet Red Bile Agar (VRBA) in which the agar only allows coliform bacteria to grow.

Serial dilution of 9 ml peptone water was prepared and label the 6 individual sterile bottles with -1, -2, -3, -4, -5 and -6, respectively. One ml of milk sample was transferred into bottle '-1' by using sterile pipette and mix thoroughly. Then by using new pipette tip, one ml was transferred from mixed content bottle '-1' into bottle '-2' and mixed gently. Repeat the step for remaining bottles. Three empty petri dish was prepared and labelled as '-4, -5, -6'. One ml of aliquot from dilution -4 to -6 was transferred to the respective labelled petri dishes. After that, sterile VRBA was added approximately 12 ml into each petri dish which was maintained at 44℃ - 45℃ water bath. Petri dishes were then swirled to evenly spread the agar and the aliquot. After solidified, invert the petri dishes, and incubate 37℃ for 24 hours. The growth of colonies is counted using the colony counter after 24 hours. The number of colony were recorded per ml sample. According to the Fifteenth Schedule (Regulation 39) in Malaysia Food Act 1983, Coliform count should be less than 5x10 per ml.

3.4.8 Milk Composition

Determination of milk composition was done by using automated machine, which is by using Milkoscan™ FT2. Firstly, the machine needs to be set up to analyse buffalo milk. About 12 ml milk sample was transferred into a beaker and put under vibrating pipette of the machine. The analysis can be done in approximately 30 seconds for each milk sample. The machine can read about 12 parameters of the milk components.

CHAPTER 4

RESULTS

4.1 Specific Gravity

The results of buffalo milk specific gravity test are shown in Table 4. The milk specific gravity for sample from farms 1, 2 and 3 are all within the normal range (1.031 to 1.038), however the specific gravity of milk sample from farm 4 was lower than the normal range.

Table 3: The specific gravity (SG) of milk samples from each farm.

Farm	SG
1	1.037
2	1.031
3	1.034
4	1.028
Normal range	1.031 - 1.038

4.2 Clot on Boiling (COB)

The result of COB is shown in Table 5. The results showed that milk samples from farms 1 and 2 were presence of precipitation after five minutes observation while samples from farms 3 and 4 have no precipitation observed.

Table 4: Clot on Boiling observation of milk samples from each farm.

Farm	Precipitation
1	Present
2	Present
3	Absent
4	Absent

4.3 Alcohol Test

The results of the alcohol test for milk samples from each farm is shown in Table 6. The results demonstrated that milk samples from farms 1 and 2 showed positive precipitation while farms 3 and 4 showed negative precipitation.

Table 5: Alcohol Test of milk samples from each farm.

Farm	Precipitation
1	Positive (Present)
2	Positive (Present)
3	Negative (Absent)
4	Negative (Absent)

4.4 Resazurin Test

The results for the resazurin test on milk samples in each farm is shown in Table 7. The test revealed that milk samples from farms 1, 3 and 4 have good to excellent quality of milk, while milk sample from farm 2 have a fair quality of milk.

Table 6: Resazurin Test grading of milk samples from each farm.

Farm	Lovibond Disk number	Grade of milk
1	4	Good
2	3	Fair
3	6	Excellent
4	6	Excellent

4.5 Methylene Blue Reduction Test (MBRT)

The results of Methylene Blue Reduction Test are demonstrated in Table 8. Milk samples from farms 1 and 2 undergo complete blue reduction in less than 5 hours indicating positive MBRT, while the milk samples from farms 3 and 4 took more than six hours to undergo complete blue reduction, indicating negative MBRT

Table 7: Methylene Blue Reduction Test for milk samples from each farm.

Farm	Hours, min	Result
1	3 hrs 30 min	Positive
2	1 hr 30 min	Positive
3	> 6 hrs	Negative
4	> 6 hrs	Negative

4.6 Total Plate Count (TPC)

The results of TPC of milk samples from each farm are shown in Table 9. The results showed that only TPC of milk sample from farm 3 was less than 1.0 x 10^5 cfu/ml. Whereas the milk samples from farms 1, 2 and 4 showed contaminations with bacteria and exceeded the limit set by the Food Regulation 1985.

Table 8: Total Plate Count (TPC) of milk samples from each farm.

Farm	cfu/ml
1	9.64 x10^6
2	8.33 x10^6
3	1.0 x10^4
4	1.1 x10^5

4.7 Coliform Test

The results of coliform test for milk samples from each farms are displayed in Table 10. The calculation was done according to U.S. Food and Drug Administration (USFDA) Bacteriological Analytical Manual (BAM) (2001). The coliform test showed that only farms 1 and 2 have exceeded the standard limit (50 per ml) which shows low quality milk, while milk samples from farms 3 and 4 showed less that the standard limit (<1.0 x104).

Table 9: Coliform test of milk samples from each farm.

Farm	cfu/ml
1	1.9 x10^6
2	2.7x10^7
3	<1.0 x10^4
4	<1.0 x10^4

4.8 Milk Composition

The results of milk composition for Fat, Protein, Lactose, Total solid, Solid Non-Fat, Casein, Urea and Citric Acid content of each farm is demonstrated in Table 11. Farm 1 have the highest values of the composition measured which are, Protein (4.42 %), Solid Non-Fat (10.53 %), Casein (3.62 %), and Urea (621 %). Farm 4 have the lowest value of Lactose (4.1 %), Solid Non-Fat (9.4 %), Casein (3.17 %), Urea (212 %). While farm 2 have the lowest value for Protein, and Citric Acid which are 3.87% and 0.1% respectively. The average value of each composition was calculated as follow: Fat (5.5 %), Protein (4.09 %), Lactose (4.45 %), Total solid (15.28 %), Solid non-fat (10 %), Casein (3.31 %), Urea (392.25 mg/100 ml), Citric acid (0.125 %)

Table 10: Milk composition in milk samples from each farm.

Farm	1	2	3	4	Average	Standard (from references)
Fat (%)	4.65	7	4.73	5.62	5.5	7.0
Protein (%)	4.42	3.87	4.1	3.96	4.09	4.0
Lactose (%)	4.65	4.39	4.67	4.1	4.45	5.1
Total solid (%)	15.05	16.62	14.68	14.77	15.28	16.7
Solid non-fat (%)	10.53	9.84	10.25	9.4	10.00	9.8
Casein (%)	3.62	3.19	3.24	3.17	3.31	3.20 ± 0.04
Urea (mg/100 ml)	621	295	441	212	392.25	17.5
Citric acid (%)	0.13	0.1	0.12	0.15	0.125	0.103

CHAPTER 5

DISCUSSION

Based on previous studies, in general, buffalo milk contains a higher proportion of all nutritional components than cow's milk. In this study, the standard value of buffalo milk composition of Murrah breed was referred to Handbook of Non- Bovine Mammals (Park and Haenlein, 2006).

This study revealed that the specific gravity of buffalo milk sample from farm 4 is 1.028 which is below than the normal range reported for buffalo milk (between 1.031 to 1.038) (Park et al., 2007), while the milk samples from farms 1, 2 and 3 were within the normal range. Another study by Yoganandi *et al*. (2014) found that the mean specific gravity for buffalo milk is 1.033 which is less than specific gravity of milk sample from farms 1, 2, and 3 in this study, but more than specific gravity of milk sample from farm 4. On the other hand, a study by Prajapati *et al*. (2017) discovered that the specific gravity for buffalo milk is range between 1.030 and 1.034 with the mean value of 1.032. In comparison with the present study, only farms 2 and 3 were within the normal range while farm 1 was higher than the normal range and farm 4 was lower than the normal range. The factors influence on milk density include the temperature of milk samples, biological variations in micelles, and milk processing conditions. The key factor known to affect milk density variation among the various milk components is the milk fat content. Milk with a higher fat content will have a lower specific gravity since milk fat is a lighter component, and vice versa.

This study also found that farms 1 and 2 were positive for Clot on Boiling (COB), Alcohol test, and Methylene Blue Reduction Test (MBRT) while farms 3 and 4 were negative for the same conducted tests. These tests proved that milk samples from farms 1 and 2 are acidic when compared to samples from farms 3 and 4. Acidification has a direct impact on the stability of casein micelles by lowering their charge, dissolving part of the insoluble calcium phosphate crosslinks, and altering internal protein bonds (Lucey, 2016), finally forming precipitation in COB and alcohol test. Milk samples from farms 1 and 2 comply with the Food Regulations 1985 stating that the sample must not completely decolourize the methylene blue solution in less than 5 hours in any reductase test. The discolouration of methylene blue is due to oxygen depletion due to high microbial activity

The Resazurin test shares the same mechanism as MRBT since the changes of resazurin dye are based on bacteria present in the milk and the leucocyte content High bacteria content will remove oxygen significantly and the resazurin dye will discolored faster. According to Nath and Aravindkumar (2021) to show good to excellent quality of milk, the number of resazurin disks must be 4 and above. The present study revealed that Lovibond Disk number for farms 1, 3 and 4 had more than 3 which indicates good to excellent quality of milk, but not from farm 2 which shows a resazurin disk number of 3.

This study shows that only farms 3 and 4 having TPC less than 1.0 x10^5 cfu/ml, however, farms 1 and 2 had exceeded the standard limit set by Malaysia Food Act (1983). Similar results obtained for Coliform Test in which farms 3 and 4 abide by the regulation in which the colony count was less than 5.0 x10 cfu/ml set by the Malaysia Food Act (1983). As study conducted by Chye (2004) found that a healthy udder of the animal, a clean milking process, sanitary equipment, and appropriate milk storage conditions can all contribute to a low TPC count.

The present study discovered that the average fat content in all milk samples to be 5.5% which is lesser than standard value of buffalo milk of 7%. Higher fat content than the present study also being reported by Misra *et al*. (2008) in which 7.53 ± 0.19% for Murrah buffalo. As comprehensive review on composition and properties of buffalo milk studied by Abd El-Salam *et al*. (2011) reported that the fat content of buffalo milk from Egypt, India, Italy, Pakistan and Brazil were all higher than the result of the present study which are (7.0 – 7.2 %), (7.7 ± 0.1 %), (8.1 ± 1.9 %), (7.6 ± 0.1%), (6.6 %) respectively. Wanapat and Kang (2013) writes in their study that typical fat content of buffalo milk is 7.90 % which are higher than the present study. While in the previous final year project (FYP) conducted in 2018 (unpublished data) reported that the average fat content of buffalo milk in Kedah was 7.22% which is higher than the present study.

The buffalo milk average protein content in the present study was 4.09%. It is slightly higher than the standard value of typical buffalo milk which is 4.0%. The present protein content is also higher than the other study reported by Misra *et al*.

(2008) which stated that the protein content was 4.03 ± 0.05% in Murrah breed. Another study conducted by Wanapat and Kang (2013) found that protein content is 4.20% which is higher than the present study. While Khedkar *et al*. (2016) discovers that the protein content of buffalo milk from Italy, Egypt, USSR and India are 3.95%, 3.87%, 4.32%, and 3.90% respectively. This is higher than the buffalo milk in Italy, Egypt and India but lower than USSR. In addition, the previous final-year project conducted in 2018 (unpublished data) reported the average protein content of buffalo milk in Kedah was 3.56 % which is lower than the present study.

The average lactose content in buffalo milk found this study is 4.45% which is lower than the standard value of 5.1%. Also, the lactose content higher than the present study also being reported by the Wanapat and Kang (2013) and Han *et al*. (2007) represented by 5.00% and 5.07 ± 0.13 % respectively. Another study by Khedkar e*t al*. (2016) discovers that the result of lactose content in buffalo milk in Italy, Egypt, USSR and India are all higher than the present study which represents by 4.88%, 5.0%, 4.96% and 5.28% respectively. In addition, the previous final-year project conducted in 2018 (unpublished data) reported that the average lactose of buffalo milk in Kedah area was 3.48 %, thus much lower than the present study.

The present study found that the average of the total solid content in buffalo milk was 15.28% which is lower than the standard value of typical buffalo milk of 16.7%. A higher average of total solid content in buffalo milk than the present study also being reported by Misra *et al*. (2008) which is 16.53 ± 0.20% for Murrah buffalo. Wanapat and Kang (2013) stated that their finding on average total solid in buffalo milk was higher than in the present study, which is 16.30 %. The average of total solid of buffalo milk studied by by Khedkar *et al*. (2016) in Italy, Egypt, USSR and India are 16.86%, 16.40%, 18.00%, 17.02%, respectively and are higher than the present study. Moreover, the previous final-year project conducted in 2018 (unpublished data) reported that the average total solid content of buffalo milk in Kedah area was 15.06%, which is slightly lower than the present study.

This study also revealed that the average of solid non-fat (SNF) in buffalo milk samples is 10%. It is higher than the standard value of 9.8%. The previous study also revealed that SNF in buffalo milk from Italy, Egypt, USSR and India are 9.64%, 10.03%, 10.00%, 9.96% respectively (Khedkar et al., 2016). Thus, the present study has more SNF content than buffalo milk in Italy and India, but less SNF compared to buffalo milk in Egypt and USSR. Lower SNF content than present study also has been reported by Misra *et al*. (2008) which is 9.00 ± 0.07% for Murrah buffalo. Furthermore, the previous final-year project conducted in 2018 (unpublished data) reported that the average SNF of buffalo milk in Kedah area was 7.83%, thus much lower than the present study.

Other than that, the average urea content in buffalo milk samples in this study was 392.25 mg/dL. This is 22-fold higher than the data reported by Abd El-Salam *et al.* (2011) which is 17.5 mg/dL. While another study by Biswajit *et al*. (2004) found that the urea content in the milk is in between 54.13 ± 0.62% and 56.40 ± 0.60% which is much lower than the present study. Lower urea content than present study also being reported by Rangel *et al*. (2013) in which ranged from 12 to 25.4 mg/dL, with a mean of 17.8 ± 4.4 mg/dL.

All casein in the buffalo milk is available in micellar state, unlike the cow milk, an average of 90-95% of the casein is in micellar state and the remaining portion is present in serum form (Khedkar *et al*., 2016). The average casein in buffalo milk samples the present study is 3.31% which is slightly higher than the average buffalo milk of Murrah buffalo reported by Misra *et al*. (2008) which is 3.20 ± 0.04%.

The present study showed an average of citric acid in buffalo milk samples is 0.125%. It is lower than the average reported in the previous study (Sherwood and Hammer, 1926) in which the reported value of citric acid in buffalo milk was 0.13 to 0.23%.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In general, this study revealed that buffalo milk possesses good quality of milk since most of the samples show good quality in most of the quality tests. However, milk samples from farms 1 and 2 showed low quality in term of microbial activity probably due to poor hygiene and management practices. While for the nutrient composition in this study, although some of the nutrient compositions are lower than the standard value stated in the Handbook of Milk of Non-Bovine Mammals by Park and Haenlein (2006) for fat, lactose, total solid, but most of the nutrient composition are higher than the standard value in term of protein, SNF, urea, casein and citric acid. Thus, this study concludes buffalo milk produced by local farmers n Selangor has good quality of milk and have good nutrient composition. Therefore, buffalo farm is worth to be commercialised and well-establish in Malaysia to improve their management system and produce more quality of milk.

To improve this study in future, it is recommended that the milk samples is replicated from each of the farm to increase the reliability of the results. By doing this, average results can be obtained to represent true quality and each component of the buffalo milk from each of the farms. Other than that, periodical analysis also can be done in which the milk samples need to be collected over some period of time to be able to evaluate or investigating the factors that affecting buffalo milk quality and milk composition. Finally, it is recommended that future study need to include a large distribution of buffalo farms in Selangor as it could really represent the whole buffalo in Selangor area.

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APPENDICES