INCIDENCE OF *MYCOBACTERIUM BOVIS* AND QUALITY ASSESSMENT IN FRESH AND FERMENTED MILK SOLD IN GOMBE METROPOLIS, GOMBE STATE, NIGERIA

BY

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DECLARATION

I hereby declare that this work is the product of my research work undertaken under the supervision of Professor M.D. Mukhtar and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged, by means of references.

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CERTIFICATION

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ABSTRACT

This study was carried out for the assessment of microbiological quality and incidence of *Mycobacterium bovis* in fresh and fermented milk sold in Gombe metropolis in the period February to July, 2016. One hundred and eighty samples were collected comprising of ninety fresh and ninety fermented milk from farms and market respectively. Total aerobic and anaerobic mesophilic bacteria plate count was conducted. Lowenstein Jensen pyruvate medium was used for the detection of *Mycobacterium bovis*. Coliform count was ascertained by multiple tube fermentation technique. Proximate analysis, assay for vitamin A, C and E and analysis of mineral elements were conducted using AOAC procedures. Analysis of variance was used to test whether the contamination level among fresh and fermented milk samples are significantly different ($P< 0.05$). The study showed a range of total aerobic plate count in fresh and fermented milk to be $5.18x10^8$CFU/mL to $8.73x10^8$CFU/mL and $6.53x10^8$CFU/mL to $9.18x10^8$CFU/mL respectively. Anaerobic count in fresh and fermented milk ranges from $2.33x10^3$CFU/mL to $2.65x10^3$CFU/mL and $1.21x10^3$CFU/mL to $3.60x10^3$CFU/mL respectively. Incidence of *M. bovis* ranges from 6(6.67%) to 21(23.33%) in fermented and fresh milk samples respectively. Furthermore, the number of coliform indicator organisms ranges from 4.93±2.3MPN/100mL to 10.07±4.9MPN/100mL and 5.97±2.1MPN/100mL to 6.17±2.4MPN/100mL in fresh and fermented milk respectively. The proximate analysis of the samples showed moisture, protein, fat, ash and carbohydrate of 13.13±19.0, 30.15±24.9, 6.41±3.6, 2.48±1.9, 47.59±20.9 mg/100mL respectively. The vitamin analysis showed vitamin A in fresh and fermented milk ranges from 156.23±19.7IU/100mL to 179.78±26.8IU/100mL and 47.48±22.1IU/100mL to 75.23±25.2IU/100mL. Vitamin C ranges from 1.59±0.3mg/100mL to 1.85±0.4mg/100mL and 0.59±0.14mg/100mL to 0.75±0.15mg/100mL in fresh and fermented milk respectively. Vitamin E ranges from 0.58±0.21mg/100mL to 0.77±0.33 mg/100mL and 0.32±0.20 mg/100mL to 0.34±0.20 mg/100mL in fermented and fresh milk samples respectively. Content of magnesium, manganese, calcium, potassium and sodium (mg/100mL) of the samples obtained was 17.07±14.7, 9.56±17.3, 251.40±64.3, 159.86±27.8, 243.69±199.6 respectively. The predisposing factors to contamination are promoted by the environment, animals and pastoralist. The milk contained physico-chemical parameters that are in-line with the internationally acceptable limit. However, in terms of microbial count of fresh and fermented milk, there is no choice. Milk vendors in Gombe leave much to be desired in terms of microbiological quality of the product. The milk is said to be safe in terms of contamination by coliform bacteria In spite of the fact that both fresh and fermented milk examined contained an appreciable mineral elements but least in manganese although within the recommended values. They also contained vitamins in good quantity of vitamin A, vitamin E and vitamin C. It can be recommended that the producers and vendors should be taught the procedures of pasteurization and adherence to the application of food safety guidelines. NAFDAC and ISO should take action to ensure supply of safe milk to public.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Milk is an opaque white liquid food drink which can be fresh or fermented. Fermented milk is called nono in Hausa, although it is called nunu by some tribes in Nigeria and it contains good qualities of amino acids, calcium, phosphorous and vitamins A, C, E and the B complex (Nebedum and Obiakor, 2007).

The nutritional composition of milk makes it not only suitable for human nutrition but also ideal for microbial life. Outbreaks of milk-borne diseases have occurred despite pasteurization, as a result of either improper pasteurization or product re-contamination (Ogbonna, 2011).

Raw milk contains microorganisms which may likely cause disease; even when the milk is fermented; the fermentation process with the attendant drop in pH may not rid the product of these organisms and may be carried to consumers (Ogbonna, 2011).

Milk has been preserved since early times by fermentation; many traditional fermented milk products were made (Akabanga, *et al.,* 2010). Fermented milk products fulfill multiple purposes in rural developing communities (O' Mahony, 1988), they are consumed as food and beverages and the markets value and storage life are improved over that of raw milk (Motarjemi and Nout, 1995).

Raw milk has low keeping quality at room temperature, spontaneous microbial spoilage occurs turning the product sour. This is brought by the activity of lactic acid bacteria (Wouters *et al.,*

2002). Depending on the preservation and the process-line, micro organisms other than lactic acid bacteria could be found in the milk (Ogbonna, 2011).

Different categories of microorganisms could be found in the milk since the udder of the animal could harbor organisms while others may come as contaminant due to poor handling (Ogbonna, 2011). Most handlers or sellers of nono are streets peddlers and not all the nono sent to the market by peddlers is sold the same day and in most developing countries such unsold ones have to go back to the market with no special attention to preservation or safety (Ogbonna, 2011).

However, consumption of milk contaminated by *Mycobacterium Bacilli* such as *Mycobacterium bovis* has long been regarded as the principal mode of transmission from animals to humans (Acha and Szyfres, 1987). *Mycobacterium tuberculosis* and *Mycobacterium bovis* are the classic agents causing human and bovine tuberculosis (TB) respectively. There have been instances of isolation of *M. tuberculosis* as well as *M. bovis* from bovine tissues and milk (Sirivastava *et al.,* 2008; Firdessa *et al.,* 2012; Gumi *et al.,* 2012).

These two tubercle bacilli can be transmitted between humans and animals in two-way fashion directly (i.e. via aerosol inhalation) or indirectly from animals to humans (i.e. by consumption of raw contaminated dairy products and meat) (CDC, 2005; Ocepek *et al.,* 2005)

Milking cows with the TB of the udder may act as amplifiers of TB by rendering the whole milk supply infective in place where milk pooling is practiced. Consumption of such food is likely to cause extra pulmonary TB (EPTB) (Solomon, 2014).

Neither the diagnosis of extra pulmonary TB nor the distinction whether TB is caused by *M. tuberculosis* or *M. bovis* is possible by chest radiography, sputum smear microscopy and sputum culture (Solomon, 2014). Misdiagnosis of *M. bovis* infection for *M. tuberculosis* or vice versa

can have important consequences including the wrong treatment with pyrazinamide in the case of the former (Solomon, 2014).

Humans can develop latent TB infection, active or reactivation of latent TB. The active TB infection is not as important as it is in human beings, since in both cases the animal must be slaughtered (Carlos *et al.,* 2012).

SD Bioline TB Ag; MPT64 Ag (SD MPT64 Rapid test), a simple immunochromatographic test (ICT) for the *M. tuberculosis* complex, has been developed and uses monoclonal antibodies to detect MPT64 protein. MPT64 is a protein specifically secreted by members of the *M. tuberculosis* complex including *M. tuberculosis*, *M. bovis*, and *M. africanum* as well as some strains of *M. bovis* bacilli Calmette-Guérin (BCG). The SD MPT64 Rapid Test is used widely to confirm the identity of *M. tuberculosis* isolates from humans. Furthermore, the test is highly sensitive and specific. The SD MPT64 Rapid test is potentially useful for identifying *M. bovis*, a member of the *M. tuberculosis* complex, because the MPT64 antigen is expressed by all members of the *M. tuberculosis* complex. However, few reports have evaluated the performance of this test for analyzing *M. bovis* isolates from animals. This test is a rapid immunochromatographic identification test for the *M. tuberculosis complex* isolates that uses mouse monoclonal anti-MPT64. Mouse monoclonal anti-MPT64 was immobilized on the nitrocellulose membrane as the capture material (test line). Another antibody, which recognized another epitope of MPT64, conjugated with colloidal gold particles was used for antigen capture and detection in a sandwich type assay (IOS EBP-DMA 007).

1.2 Justification for the Study

Despite control measures taken during fermentation of milk, contamination of the milk by *M. bovis* may occur due to the poor handling of the milk and it may lead to the bovine tuberculosis disease. However contamination may also occur as a result of shedding of the organisms from the udder of the cow during milking and may be contaminated during fermentation. *Mycobacterium bovis* may be found in milk which if not carefully treated can cause infection (Danbirni *et al.,* 2010).

Unhygienic practices and lack of clean environments can lead to milk contamination and poor pasteurization of milk may allow harmful bacteria like *Mycobacterium bovis* to be consumed in milk; this could lead to serious consequences. Therefore, there is need to carried out this research to elucidate factors that could make milk and nono consumption safe.

1.3 Null Hypothesis

The null hypothesis is that there should be no association between *Mycobacterium bovis* with milk since it comes pure in its natural state. However, there is a question that milk can contain *Mycobacterium bovis* because of the suspicion of poor handling during milking and also during fermentation of the milk.

1.4 Aim of the Study

The aim of this study is to investigate the microbiological quality and also to screen for the presence of *Mycobacterium bovis* in fresh and fermented milk sold in farms and markets in Gombe metropolis, Nigeria between February and July 2016.

1.5 Objectives of the Study

The specific objectives of the study where to;

1. Identify some of the predisposing factors associated with contamination of milk at the collection site by personal observation.
2. Enumerate total aerobic and anaerobic mesophilic bacterial counts in the samples.
3. Screen the milk samples for contamination with *Mycobacterium bovis*.
4. Enumerate the total coliform bacteria in the milk samples.
5. Determine the nutritional composition of the milk samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Source of Milk Contamination

Milk contamination by micro organisms generally occurs from three main sources; from within the udder, from the exteriors of the udder, and from the surfaces of milk handling and storage equipments (Bramley and Mckinnon, 1990). Moreover, Murphy and Boor (2000) reported that the health and hygiene of the cow, the environment in which the cow is housed and milked, and the procedures used in cleaning and sanitizing the milking and storage equipment, all influence microbial numbers in milk. They added that, temperature and length of storage time are important that may allow microbial contamination to reproduce.

Furthermore, in the market milk is exposed to various contaminants due to the nature of the environments. Most of the nono markets if not all in the developing countries are not floored and the vendors sell their products (fresh or fermented milk) in the sand which is another possible source of milk contamination. The vendors do not take adequate measured to ensure the safety of their milk; they always used the same container to measure the milk from their calabash when someone is buying the milk and they hold the container from every angle. They can open the milk and leave it open and this allows flies to enter and contaminate the milk (WHO, 2006).

2.2 Bovine Tuberculosis

Bovine tuberculosis is a generally chronic respiratory disease, which is clinically difficult to diagnose although emaciation, loss of appetite, chronic cough and other signs of pneumonia could be symptoms developing at relatively late stages of the infection in cattle (Ayele *et al.,* 2004). Especially in developing countries, clinical forms of many other chronic, emaciating diseases, like African trypanosomiasis, chronic contagious bovine pleuro-pneumonia (CBPP) or chronic multiparasitism, are difficult to be distinguished from bovine tuberculosis. Bovine tuberculosis pathology is characterized by the formation of granulomatous lesions, which can

within the course of the disease progress or exhibit extensive necrosis, calcify or liquefy and subsequently lead to cavity formation (Cassidy, 2006; Hope and Villareal-Ramos, 2008). During meat inspection procedures on cattle carcasses in slaughter houses, tuberculous lesions are primarily found in the upper and lower respiratory tract and associated lymph nodes (Cassidy, 2006).

However, the bacteria can also develop a systemic infection, disseminate within its host and affect other organs (Coetzer and Tustin, 2004). As all *Mycobacterium* spp., *M. bovis* has an unusual cell wall surface structure characterized by the dominant presence of mycolic acids and a wide array of lipids (Glickman *et al.,*, 2001). This waxy lipid envelope confers an extreme hydrophobicity, which renders the bacteria acid- and alcohol-fast, a feature that can be exploited to identify mycobacteria via the Ziehl-Neelsen staining technique (Steingart *et al.,* 2003). The mycobacterial surface lipids also have a potent biologic activity and are thought to play a crucial role in pathogenesis (Glickman *et al.,* 2001).

M. bovis can be identified on the basis of specific biochemical and metabolic properties. E.g., *M. bovis* requires pyruvate as a growth supplement, is negative for niacin accumulation and nitrate reduction, shows microaerophilic growth on Lebek medium and is generally resistant to pyrazinamide. In contrast, *M. tuberculosis* does not require pyruvate as a growth supplement, is positive for niacin accumulation and nitrate reduction, shows aerophilic growth on Lebek medium, and is usually not mono-resistant to pyrazinamide (Cole, 2002; Kubica *et al.,* 2006). However, the unequivocal validity of these characteristics is challenged by several studies (Niemann, *et al.,* 2000; Kubica *et al.,* 2006). Different molecular markers and techniques have been discovered and developed in the past that allow the unambiguous identification and

differentiation of *Mycobacterium* spp. and the members of the MTBC (Huard *et al.,* 2006; Pinsky and Banaei, 2008).

Milk production has increase in most developing countries as consequences of greater demand for milk for human consumption (FAO, 1993). In Nigeria, despite increased importation of exotic diary breeds of cattle to meet up with the demand for increased in milk production, the nomadic pastoralist indigenous zebu cattle constitute the larger proportion of dairy cattle (Ariyo, 2002).

In those areas where extensive management is more common, animal crowding (near watering ponds, dip tanks, markets and corals) still plays major roles in the spread of disease (Ayele *et al.,* 2004). Milk obtained through hand milking from such infected cow is either taken as fresh cow milk or fermented (nono) without due processes of grading, classification and pasteurization. These unhygienic practices have led to the isolation of *M. bovis* in fresh raw and fermented nono in various parts of the country (Idrisu and Schnurrenberger, 1977; Abubakar, 2007). In industrialized countries, bovine control and elimination programme together with milk pasteurization have drastically reduced the incidence of the disease caused by *M. bovis* in both cattle and humans (Danbirni *et al.,* 2010). However, in developing countries, bovine is widely distributed and control measures are either not applied or applied sporadically and pasteurization of milk is rarely practiced. This is because of financial constraints; lack of political will as well as limitation of diagnostic test in detecting early exposure before the bacilli begins to shed in milk secretion to susceptible animals and humans (Cosivi *et al.,* 1998).

2.3 Etiology

Mycobacterium bovis is a slow growing, facultative intracellular, aerobic and gram-positive bacterium with a dysgonic colony shape when cultured on Löwenstein-Jensen medium (Kubica *et al.,* 2006).

Mycobacterium bovis is a member of the *M. tuberculosis* complex (MTC), a group of species consisting of *M. bovis, M. africanum, M. microti, M. caprae***,** *M. pinnipediie, M. mungi, M. canetti,* all with a high genetic homology. *M. bovis* is the etiological agents of bovine tuberculosis (bTB) which causes economic and public health problems in many countries (Carvalho, *et al.,* 2014).

2.4 Mode of Transmission

Airborne infection is the most common transmission route and more than 19% of cattle with bovine shed the *Mycobacteria* mainly early in the course of the infection (Alvarez *et al.,* 2009). Studies with molecular markers have shown that infected cattle are a potential source of tuberculosis transmission to humans (Serano-moreno *et al.,* 2008). *M. bovis* has been isolated from milk storage tanks, inadequately pasteurized milk and from tuberculin non-reacting cattle (Pardo *et al.,* 2001; Leite *et al.,* 2003).

2.5 Pathogenesis

Bovine tuberculosis is spread from animal to animal and sometimes to human attendants in moist coughs spray. About 1% of the infected cows develop lesions in the udder and bacilli are excreted in the milk which can then infect people who drink it raw (Arora and Arora, 2012).

M. bovis enters the mouth as in milk-borne bovine tuberculosis, the primary complexes involve the tonsil and cervical lymph nodes or the intestine, often the ileocaecal region and the mesenteric lymph nodes (Arora and Arora, 2012).

Inhaled tubercle bacilli are engulfed by the alveolar macrophages which they replicate to form the initial lesion or Ghon focus. Some bacilli are transported by macrophages to the hilar lymph nodes to forms the primary complex (Arora and Arora, 2012).

Human tuberculosis is divisible into primary and post-primary (secondary) forms. The primary tuberculosis begins with inhalation or ingestion of the *Mycobacteria* and ends with T cell-mediated immune response that induces hypersensitivity to the organisms and control 95% of infection. The post-primary (secondary) tuberculosis is cause by re – activation of the primary lesion (endogenous) or by exogenous re – infection (Arora and Arora, 2012)

2.6 The Burden and Epidemiology of *Mycobacterium bovis* Infections

2.6.1 The Burden of Bovine Tuberculosis

Bovine tuberculosis is primarily of economic importance as it can have a considerable direct effect on milk and meat production and animal reproduction (Zinsstag *et al.,* 2006). Moreover, national and international trade and other economic sectors may be indirectly affected by the disease (Zinsstag *et al.,* 2006). Bovine tuberculosis can also infect wildlife and thus have unpredictable consequences for entire ecosystems. E.g., in the southern region of the Kruger National Park in South Africa, 38% of the buffalos are infected with strains of *M. bovis*, originally introduced from domesticated cattle (Renwick *et al.,* 2006). Carnivores such as lions, cheetah and leopards, feeding on infected animals are frequent spillover hosts (Renwick *et al.,* 2006). Moreover, wildlife reservoirs of *M. bovis* hamper disease eradication schemes in several countries (Bengis *et al.,* 2002 and Michel *et al.,* 2006).

Bovine tuberculosis also bears a zoonotic potential and it is of public health concern (WHO, 1994 and Theon *et al.,* 2006). Infections of humans with *M. bovis* are rare in most countries that

successfully apply disease control measures. However, zoonotic transmission of *M. bovis* may be frequent in countries where the disease is enzootic in cattle.

In a recent study in the San Diego region of California, USA, *M. bovis* infections accounted for 45% of the tuberculosis cases in children (Rodwell *et al.,* 2008); most of these children were of Hispanic origin with ties to Mexico, where bovine is prevalent. Importantly, persons with *M. bovis* infections were 2.6 times more likely to die during treatment than persons with *M. tuberculosis* infections (Rodwell *et al.,* 2008).

The poor, especially in developing countries, are thought to be at highest risk to contract zoonotic tuberculosis (WHO, 2006) and also the observed higher susceptibility of HIV-infected persons to *M. bovis* infections is of major concern (Lobue, 2006). The problems associated with bovine are of particular importance for many countries in Africa and especially the arid and semi-arid zones, where more than 50% of all African cattle, sheep and goats are raised and where the livelihood of millions of people is based on livestock farming (WHO, 2006).

According to Thornton *et al.,* there are an estimate of 556 million poor livestock keepers in the world with 30% of them living in sub-Saharan Africa and being most severely affected by the consequences of bovine (Thornton *et al.,* 2002; WHO, 2006). However, data on disease prevalence in cattle or wildlife or the frequency of zoonotic transmission is generally scarce. This is mostly due to the absence of disease surveillance, insufficient laboratory capacity and the lack of veterinary expertise and may lead to a general underestimation of the disease prevalence in these regions (Ayele *et al.,* 2004).

2.6.2 Bovine Tuberculosis Epidemiology

M. bovis can infect most mammalian species although bovids and especially cattle are the main hosts (Kaneene and Pfeiffer, 2006). Transmission between animals is mostly thought to occur by

inhalation of contaminated aerosol (Kaneene and Pfeiffer, 2006). Evidence for a generally respiratory route of infection has first come from studies of the tuberculous lesion distribution in cattle. Most commonly, the upper and lower respiratory tract and associated lymph nodes are affected by lesions and the minimum dose required to establish disease in artificially infected cattle appeared to be 1000 times less for the respiratory route than for the oral route (Cassidy, 2006).

However, infection can also occur via the gastro-intestinal tract when animals ingest contaminated food, water or milk. In this case, lesion distribution is characterized by the presence of mesenteric lymph node lesions (Kaneene and Pfeiffer, 2006).

In a study in Ethiopia, mesenteric lymph node lesions were more often found in grazing animals compared to animals kept indoors (Ameni *et al.,* 2006). Therefore, infection via the gastro-intestinal tract may be more important in cattle subjected to extensive livestock production systems as they are commonly observed in Africa. Because close contact between animals is relatively rare in extensive production systems, aerosol transmission of *M. bovis* is most likely occurring at water points, or when animals are gathering at night for protection from predators or during the daytime under trees when seeking shelter from the sun (Ayele *et al.,* 2004). Different routes of transmission may take place if tuberculosis infection becomes systemic and other organs such as the urinary tract or the mammary glands become involved (Coetzer and Tustin, 2004; Kaneene and Pfeiffer, 2006). Especially the latter can be responsible for early infections in calves (Kaneene and Pfeiffer, 2006). Moreover, consumption of contaminated milk represents the most important route of zoonotic tuberculosis transmission although disease communication can be easily prevented by milk pasteurization (Cosivi *et al.,* 1998; Ayele *et al.,* 2004).

Commonly found risk factors for tuberculosis disease in cattle are close contact of animals such as in intensive livestock production systems (Cosivi *et al.,* 1998; Ayele *et al.,* 2004; Ameni *et al.,* 2006 and Cleaveland *et al.,* 2007), increasing herd size (Cleaveland *et al.,* 2007) and increasing age (Kazwala *et al.,* 2001; Oloya *et al.,* 2006 and Munyeme *et al.,* 2008;). Another important risk factor is the contact or proximity of cattle and wildlife reservoirs of *M. bovis*, which obstruct disease eradication schemes in a number of countries. E.g., in the UK the Eurasian badger (*Meles meles*) represents a maintenance host for *M. bovis* (Cheeseman *et al.,* 1989). White-tailed deer (*Odocoileus virginianus*) has been identified as a disease reservoir in Michigan, USA (O'Brien *et al.,* 2008), the brush tail possum (*Trichosurus vulpecula*) is a disease reservoir in New Zealand and the African buffalo (*Syncerus caffer*) has been identified as a main reservoir host for *M. bovis* in Southern Africa (Michel *et al.,* 2006). Recent work also indicates an important influence of host genetics on disease susceptibility.

In this respect, a report from Ethiopia described lower disease prevalence in African cattle breeds compared to exotic animals (Ameni *et al.,* 2006; Ameni *et al.,* 2007); but also differences between distinct zebu breeds have been described in Chad (Diguimbaye-Djaibe *et al.,* 2006). The main risk factors for *M. bovis* infections in humans are poverty, malnutrition, HIV infection, the consumption of raw milk and close contact to livestock (Cosivi *et al.,* 1998, Ayele *et al.,* 2004).

M. bovis causes less than 1.4 percent of pulmonary tuberculosis cases outside Africa. Within Africa *M. bovis* causes approximately 2.8 percent of cases of pulmonary tuberculosis for a crude incidence of seven cases per 100,000 population (Muller *et al.,* 2013).

The global proportion of *M. bovis* is higher among patients with extra pulmonary tuberculosis because the pathogen is frequently acquired via oral ingestion and gastrointestinal disease is an important clinical manifestation (O' Reilly and Daborn 1995).

In developed countries, where *M. bovis* in cattle is controlled and dairy products are routinely pasteurized, the proportion of *M. bovis* infection among human tuberculosis cases is often lower than the global estimate (HPA, 2008). The annual incidence of *M. bovis* decreased from 0.065 to 0.047 per 100,000 populations (Mandal *et al.,* 2011).

2.7 Nature of Cell Wall

The cell wall consists of lipids, proteins and polysaccharides. Lipid contents account for 60% of the cell wall weight. Lipids of the cell wall particularly the mycolic acid fraction are responsible for:

1. The hydrophobic character of the organisms which tends to adhere to each other during growth in aqueous media and to float at the surface unless disperses with detergents.
2. Relative permeability to stains.
3. Acid fastness.
4. Unusual resistance to killing by acids and alkalis.
5. Resistance to bactericidal action of antibodies and complement.
6. Slowness of growth by hindering permeation of nutrients into the cell.
7. Cellular tissue reactions of the body.

The cell wall is made up of four layers. The innermost layer is Peptidoglycan followed by arabinogalactan layer, mycolic acid layer and mycosides (Arora and Arora, 2012)

2.8 Immunology of Bovine Tuberculosis

Based on the predominance of tuberculous lesions in the respiratory tract and associated lymph nodes of diseased cattle, it was early believed, that bovine tuberculosis is transmitted from

animals to animals through inhalation of infectious aerosols (Cassidy, 2006). Further evidence for this route of transmission has come from a number of other studies (Cassidy, 2006).

Bacteria entering the respiratory tract, passing the mucociliary layer and gaining access to the alveolar space are thought to be phagocytized by macrophages, which may constitute the main cellular host for *mycobacteria in vivo* (Hope and Villareal-Ramos, 2008). Following uptake into the phagosome, macrophages attempt to kill the bacteria by phago-lysosome fusion and acidification. However, *mycobacteria* are able to prevent lysosomal delivery by manipulating the host cell signal transduction pathways using an array of bacterial effector lipids and proteins (Nguyen and Pieters, 2005).

Mycobacteria are therefore believed to reside and multiply primarily within the phagosomes before they eventually destroy the phagocytes. However, this assumption has been challenged by a recent study of van der wel *et al.,* describing the translocation of *M. tuberculosis* from phago-lysosomes to the cytosol of myeloid cells, from the second day after phagocytosis (Van der *et al.,* 2007). Although infected macrophages are not believed to act as the main antigen presenting cells (APC) they can trigger an immune response through the secretion of pro-inflammatory cytokines (e.g. TNF-α) and chemokines, leading to the recruitment of other phagocytes and lymphocytes to the lung (Theon and Barletta, 2006; Hope and Villareal-Ramos, 2008).

The most important APCs in tuberculosis infection are probably dendritic cells, which also play a major role in modulating the host immune response. *Mycobacteria* may get access to the lung tissue through M-like cells in the bronchi. From there, they can penetrate to the underlying lymphoid tissues, get phagocytized by dendritic cells and transported to the draining lymph nodes, where immune responses are initiated (Hope and Villareal-Ramos, 2008). The interaction

of *M. bovis* with bovine dendritic cells leads to cell maturation and increases expression of surface molecules involved in T-cell interactions (Hope *et al.,* 2004).

Moreover, altered cytokine profiles and especially the secretion of IL-12 and TNF-α are observed upon infection of bovine dendritic cells with *M. bovis* (Hope *et al.,* 2004). The secretion of these cytokines and mycobacterial antigen presentation on the surface of dendritic cells help triggering an adaptive cell mediated immune (CMI) response. This CMI response is commonly known as the Th1-type immune response and characterized by the secretion of high levels of IFN-γ and IL-2 by Th1 CD4 T-cells (Theon and Barletta, 2006). The production of IFN-γ and IL-2 by Th1 cells can activate macrophages in order to become highly microbiocidial. Following activation, macrophages may be able to kill most of the *mycobacteria* within the phagosome by releasing increased amounts of hydrolytic enzymes, reactive oxygen intermediates and reactive nitrogen intermediates including nitric oxide (Theon and Barletta, 2006; Hope and Villareal-Ramos, 2008).

Interestingly, activation of bovine dendritic cells enhances their ability to kill ingested *M. bovis* to a lesser extend and significant numbers of live bacilli are able to persist. Dendritic cells may therefore constitute a reservoir for pathogenic *mycobacteria*. However, in a non-activated state, dendritic cells are more potent in killing ingested bacteria than macrophages (Hope and Villareal-Ramos, 2008).

Natural Killer (NK) cells and γδ T-cells are crucial in the early innate immunity against mycobacterial infections. They release IFN-γ when activated through the interaction with dendritic cells and thus contribute to the Th1 biased immune response. Conversely, NK and γδ T-cells also play a role in fully activating dendritic cells. Control of infection is ultimately dependent on the induction of a strong CMI response and granuloma formation, in which $CD^{+}4$

but also $CD^{+}8$ T-cells play a pivotal role. If the immune response against mycobacterial infection is strong enough to contain bacterial growth, active disease does not develop although infection may never be fully cleared. If the balance between the host's defenses and the persisting *mycobacteria* is tipped in favor of the pathogen, active disease occurs and the granuloma formation progresses. At a late stage, bovine tuberculosis granulomas are characterized by extensive necrosis which can lead to liquefaction and cavity formation. Rapture of these cavities into the bronchi consequently allows aerosol spread of the bacteria (Theon and Barletta, 2006). In a study of Cassidy *et al.,* microscopic lesions were observed in experimentally infected cows as early as seven days after inoculation of *M. bovis* (Cassidy *et al.,* 1998). Gross lesions were detected in the upper respiratory tract, in the lungs and the lymph nodes draining these areas at 14 days post-infection (Cassidy *et al.,* 1998).

At the slaughterhouse, granulomatous lesions are most often detected in lymph nodes (especially mediastinal or bronchial). This is due to the fact that fluids in an animal together with activated macrophages eventually pass through the lymph nodes where the pathogens are filtered (Theon and Barletta, 2006).

Although necessary for protection, the CMI response in tuberculosis infection can also contribute to the immuno-pathogenesis of tuberculosis. The Th2-type immune response functions as a regulatory element to counteract and down regulate the pro-inflammatory CMI response, elicited by IFN-γ producing cells (Hope and Villareal-Ramos, 2008). It is also associated with an increased humoral immune reaction and is mainly triggered by CD4 Th2 cells and the secretion of IL-4. It is believed that Th1-type immune responses wane towards late disease stages. In contrast, humoral Th2-type immune responses increase as disease and pathology progresses and bacterial load increases (de la Rua-Domenech *et al.,* 2006). Although the factors that trigger the

transition from a Th1- to a Th2- type immune response are not well known, it is thought that the CMI response is most relevant to determine protection; in contrast, the humoral response is considered detrimental (Theon and Barletta, 2006).

Furthermore, the two immunologic responses, antituberculous immunity and tuberculin hypersensitivity develop simultaneously in naturally infected host. Both these are mediated by T-cells sensitized to bacterial antigen (Arora and Arora, 2012). Humoral immunity appears to be of no relevance in tuberculosis and antibodies do not influence the course of disease.

In a non-immune host, the bacilli are able to multiply inside phagocytes and lyse the host cell while in immune host, CD4+ helper T cells and CD8+ suppressor T cells are produced. The former secrets interferon gamma which activates macrophages to kill intracellular *Mycobacteria* and the latter kill the macrophages that are infected with bacteria (Arora and Arora, 2012).

2.9 Vaccination Against *M. bovis* Infections

The development of a cattle vaccine against *M. bovis* infection has been considered a major priority for the control of bovine in the UK (Krebs *et al.,* 1998). Provided an effective vaccine is available, cattle vaccination may be the most cost effective bovine control strategy and therefore also especially useful for interventions and disease control in developing countries. The only currently available vaccine against tuberculosis in animals and humans is *M. bovis* BCG, which was developed through multiple passage of a strain of *M. bovis* on glycerol soaked potato slices (Hope and Villareal-Ramos, 2008).

However, BCG is not satisfactorily efficient in preventing disease and generally shows a variable efficacy. This variability has been attributed to several factors such as the vaccine strain itself, the regionally predominant infecting strains (Abebe and Bjune, 2006) or host genetics (Hope and Villareal-Ramos, 2008).

A particular obstacle of BCG is its sensitization of cattle to the tuberculin skin test and Bovigam®, making the discrimination of infected and vaccinated animals (differential diagnosis) impossible (Hewinson *et al.,* 2006). Current research in development of vaccines and new diagnostic tools must therefore be conducted in a coordinated manner to consider all these influencing factors in a pragmatic way (Hewinson *et al.,* 2006).

Some recent studies could show an increased protective capacity upon administration of newly developed vaccine candidates compared to BCG (Hope and Villareal-Ramos, 2008). A heterologous prime-boost strategy in cattle using a cocktail of three DNA vaccines for priming and BCG for boosting showed a better protection than BCG alone (Skinner *et al.,* 2003). A similar approach using BCG for priming and boosting with modified vaccine virus Ankara (MVA) expressing the mycobacterial antigen Ag85A showed very promising results in humans.

Another strategy, which may offer increased protection, is the neonatal vaccination of calves. This approach has shown to enhance the Th1 bias of the immune response and circumvents the potential problems caused by pre-exposure to environmental *Mycobacteria*, which may be of considerable importance in Africa (Hope *et al.,* 2005 and Hewinson *et al.,* 2006).

2.10 Distribution and Prevalence of Bovine Tuberculosis in Sub-Saharan Africa

Bovine Tuberculosis has been largely eradicated in the industrialized world with the exception of a few countries in which the presence of a wildlife reservoir obstructs disease elimination even though extensive control measures are applied (Kaneene and Pfeiffer, 2006). However, it is well known that bovine Tuberculosis is present in many developing countries (Cosivi *et al.,* 1998).

Nevertheless, due to the absence of disease surveillance and control, little accurate information is available on the prevalence and distribution of *M. bovis* infections. Within a welcome Trust funded project, we have recently established a network of scientists and veterinary authorities

from major livestock producing countries in Africa in order to discuss the problem of bovine tuberculosis and promote appropriate interventions. The first workshop with participants from West Africa was conducted in June 2007 in Bamako, Mali, where the representatives of each country also conveyed detailed information on the situation of bovine Tuberculosis in their country (Borna Ivan Muller and Matzendorf, 2010). Ayele *et al.*, (2004) and Hlavsa *et al*., (2008) reported that approximately 1.4% of human tuberculosis cases in the developed countries and 3.1% in the developing countries, respectively, could be attributed to *M. bovis*. It is also noticeable that not only *M. bovis,* but also *M. africanum* was isolated from milk of cows. It is known that the latter species is more related to *M. bovis* than *M. tuberculosis*, but it remains unclear whether it is also transmitted among cows, or whether this is an accidental transmission of *M. africanum* from humans to cows as reported several times for *M. tuberculosis*. However, the presence of *M. africanum* in milk hints at a disseminated infection in the cow, and this is to our knowledge, not reported for this bacterium.

2.11 Distribution and Prevalence of Bovine Tuberculosis in Nigeria

In a survey conducted in Ibadan by Cadmus *et al.,* strains of MTBC isolated from human tuberculosis patients and from slaughter cattle carcasses were subjected to molecular typing (Cadmus *et al.,* 2006). Of altogether 60 MTBC strains isolated from humans, three (5%) were identified as strains of *M. bovis*, six (10%) as strains of *M. africanum* and the rest as *M. tuberculosis*. Of 17 MTBC strains isolated from cattle, two were identified as *M. tuberculosis*, with one of them showing a spoligotype pattern that was also detected in strains of *M.*

tuberculosis isolated from humans. Altogether, 15 of 17 strains from cattle were identified as *M. bovis* and the spoligotype patterns of all these strains characteristically lacked spacer 30 in the standard spoligotyping scheme (Kamerbeek *et al.,* 1997). One of the spoligotype patterns detected in strains from cattle was also found in a strain of *M. bovis* from humans, thus indicating the zoonotic transmission of strains of *M. bovis* from animals to humans in Nigeria (Cadmus *et al.,* 2006). Evidence for zoonotic transmission of *M. bovis* has also come from other studies (Mawak *et al.,* 2006 and Zinsstag *et al.,* 2006).

The prevalence in cattle has been assessed by a number of studies in several regions using different methods; it ranged between 1% and 13% but appeared to be higher in the southern area of the country (S.I.B. Cadmus, pers. comm.). *Mycobacterium* spp. was also successfully isolated from milk (Zinsstag *et al.,* 2006).

2.12 Economics of Bovine Tuberculosis

Bovine tuberculosis affects the national and international economy in different ways. The most obvious losses from bovine tuberculosis in cattle are direct productivity losses (reduced benefit), which can be categorized into slaughter and "on-farm" losses (Gilsdorf *et al.,* 2006).

Slaughter losses comprise the cost of cattle condemnation and retention, with the loss from condemnation being essentially the purchased value of a slaughter animal and the loss from retention being a fraction of the value of a carcass. On-farm losses comprise the losses from decreased milk and meat production, the increased reproduction efforts and replacement costs for infected cattle (Gilsdorf *et al.,* 2006).

Effects of bovine tuberculosis on cattle productivity have been previously reviewed by Zinsstag *et al.,* Early studies in Germany estimated a decrease in milk and meat productivity in totally infected livestock of 10% ± 2.5% and 4% ± 2%, respectively (*Zinsstag et al.,* 2006). Similar milk

productivity losses were also estimated in studies from Canada, Spain and the U.S. (Gilsdorf *et al.,* 2006; Zinsstag *et al.,* 2006 and Bernues *et al.,* 1997).

The study in Canada also estimated the reproduction losses to one fewer calf in infected cows; the replacement losses were estimated at 15% for infected animals (Zinsstag *et al.,* 2006). Gilsdorf *et al.,* assumed a 20% reduction in calf weight and a 5% replacement cost for their cost effectiveness analyses in the U.S. More figures and references are given in the studies of (Zinsstag *et al.,* 2006 and Bernues *et al.,* 1997). Apart from direct productivity losses, bovine tuberculosis has profound economic consequences for national and international trade. On an international scale, bovine tuberculosis affects access to foreign markets due to import bans on animals and animal products from countries where the disease is enzootic. This situation has also major implications for other economic sectors, which are linked to livestock production. Moreover, bovine tuberculosis can create inefficiencies in the world market as e.g. economically inefficient but disease free exporting countries will receive more revenues than economically efficient countries, which cannot export animal products due to enzootic bovine tuberculosis (Gilsdorf *et al.,* 2006).

Presence of the disease in wildlife has considerable economic consequences. Not only is disease eradication more difficult and costly but bovine tuberculosis can theoretically affect entire ecosystems with unpredictable impact on many areas of private interest such as e.g. tourism (Munag'andu *et al.,* 2006 and Zinsstag *et al.,* 2006). Finally, bovine tuberculosis has a zoonotic potential and can cause disease in humans. Depending on the rate of zoonotic transmission this can have important effects on the public health sector; Borna Ivan Müller and Matzendorf (2010).

2.13 Risk Factors

Numerous risk factors for bovine tuberculosis have been identified in cattle around the world. These risk factors include a variety of parameter in relation to wildlife cattle contacts, movements, density of animals, etc (Jackson *et. al.,* 1995). Furthermore, bovine tuberculosis transmission cycle underlying failure to eradicate *M. bovis* in cattle in some areas remains poorly understood and several transmission hypotheses have been formulated they include: inadequate control measures, agro environmental factors, latency, wildlife reservoirs and movement of infected animal (Gilbert, 2005).

Bovine tuberculosis control programs are an economical burden, national animal health authorities are considering down scaling current control measures e.g., cancelling testing at purchase and reducing herd testing. Nevertheless, animal movements were shown to be risk factors in some countries, such as United State (Gilbert, 2005 and Gopal, 2006). Before these reductive measures are applied, it therefore seems appropriate to investigate the true risk represented by animal movement (Humblet, 2010).

2.14 The Nutritional Value of Milk

Milk has been part of the human diet for millennia and is valued as a natural and traditional food. Milk and dairy foods are considered to be one of the main food groups important in a healthy balanced diet, and as such feature in the majority of national food-based dietary guidelines from the British Eatwell and Australian plate model to the Chinese Pagoda and the Japanese Spinning top, the US pyramid, the Guatemalan pot and many others. As milk provides a substantial amount of vitamins and minerals in relation to its energy content, it is considered a nutrient dense food (Drewnowski, 2010).

Cow milk provides a wide range of essential nutrients to the diet. Milk is often recognized as a source of calcium and it is perhaps less commonly known that milk and milk products are also an important source of good quality protein, vitamin and minerals (FSA, 2002).

2.15 Nutrients in Milk

2.15.1 Vitamins

Vitamins are a well-known group of compounds that are essential for human health. These compounds can be classified into two main groups, water- and fat-soluble. Health problems can be present when these vitamins are either lacking or in excess. Our intake of vitamins depends on our diet. However, even foods that contain the necessary vitamins can have reduced vitamin content after storage, processing, or cooking. Therefore, people take vitamin tablets and/or consume milk-based products such as infant formula, adult formula, milk, yogurt, and cheese to supplement or incorporate these nutrients into their diet (Zhang *et al.,* 2005).

Vitamin A is important for normal vision, gene expression, growth and immune function by its maintenance epithelial cell function (Achikanu *et al.,* 2013).

Vitamin C, the L-enantiomer of ascorbic acid, is a water-soluble vitamin used by the body for several purposes. Most animals can synthesize their own vitamin C, but some animals, including primates, guinea pigs, and humans, cannot. Vitamin C was first isolated in 1928, and in 1932 it was proved to be the agent, which prevents scurvy. As a participant in hydroxylation, vitamin C is needed for the production of collagen in the connective tissue. Some tissues have a greater percentage of collagen, including: skin, mucous membranes, teeth, bones. Vitamin C is also required for synthesis of dopamine, noradrenaline and adrenaline in the nervous system or in the adrenal glands. It is a strong antioxidant (Rasanu *et al.,* 2005).

Vitamin E is a collective term for tocopherols and tocotrienols, which are natural antioxidants that prevent the rancidity of oils during storage and thus delay its shelf-life. Whereas α-tocopherol (5,7,8-trimethyltocol) is the most active form of vitamin E in vivo, α-tocopherol (7,8-dimethyltocol) is the most active in vitro (Gimeno *et al.,* 2000).

2.15.2 Mineral Elements

Milk is an excellent source of calcium which, is commonly recognized, as essential for the healthy growth and maintenance of teeth and bones. Calcium is also important for normal blood coagulation, normal energy yielding metabolism, normal muscle and nerve function, normal digestive function and normal regulation of cell division and differentiation (EFSA, 2006).

Milk and milk products are the main sources of calcium in pre-school school children and adolescents (4-18 years), adults (19-64 years) and the elderly (65 years and over) (Finch, 1998 and Gregory, 2000). A 200ml glass of semi-skimmed milk can provide a 6-year-old child with over half (55%) of his or her calcium requirement and can provide an adult (19-64 years) with over a third (35%) of his or her daily calcium requirement (FSA, 2002).

Potassium is important for helping to maintain normal blood pressure and helps maintain muscular and neurological function (EFSA, 2006). Milk and milk products are the main sources of potassium in the UK diets of pre-school children and the elderly (65 years and over), (Gregory, 2000; Finch 1998). A 200ml glass of semi skimmed milk will provide a child of 6 years with 29% of their daily requirement for potassium and an adult (19-50 years) with 9% (FSA, 2002).

Milk is also a good source of the mineral iodine. Iodine is required for the production of the thyroid hormones and normal thyroid function, for normal energy yielding metabolism and contributes to the maintenance of normal skin. A glass (200ml) of semi-skimmed milk will

provide a child of 6 years with 96% of their daily requirement for iodine and an adult (19-50 years) with 44%, but there is some seasonal variation in the iodine content of milk (FSA, 2002).

2.15.3 Proteins

Proteins are an extremely important class of naturally occurring compounds that are essential to all life processes. They perform a variety of functions in living organisms ranging from providing structure to reproduction. Milk proteins represent one of the greatest contributions of milk to human nutrition (O' Connor, 1995). There are two distinct types of proteins in milk, they are:

1. Casein
2. Whey

Caseins make up over 80% of the total protein content. Caseins do not have an organized structure, thus they cannot be denatured by heating. The casein molecules form polymers containing several identical and different molecules. Some molecules have hydrophilic regions and some have hydrophobic regions. The polymers are made up of many individual molecules and form into casein micelles. They are also formed into a colloidal solution, which gives skim milk its whitish-blue color. The amino acids in casein have hydrophobic and hydrophilic regions, allowing the caseins to act as highly effective surface active agents. In other words, caseins act as stabilizers of foams and emulsions (FSA, 2002).

Whey proteins are globular proteins which may be denatured when heated above temperature of 65^0C. The major components of whey are beta lactoglobulin and alpha lactalbumin. Whey is the liquid remaining after milk has been curdled and strained during the manufacture of cheese. It is used to produce ricotta and brown cheeses and is an additive in many processed foods, such as breads, crackers, pastries, and animal feed. Many of the important nutrients that comprise milk

are partitioned into whey. It contains many important nutrients, such as lactose, water soluble vitamins, and most of the minerals, with the exceptions of considerable calcium and phosphorus which go with the casein into the cheese curd (FSA, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study area (fig 3.1) is Gombe metropolis in Gombe State which is located on longitude 11^0 10'E and 10^0 15'N of green witch meridian above sea level with the Sudan savannah ecological zone of Nigeria. The mean annual rainfall ranges from 600mm-1200mm per annum and the maximum and minimum temperature of 22.7^0 C and 33.5^0 C respectively. The vegetation cover is open savannah wood land with trees up to six meters or more. Gombe Local Government Area is bounded in the north by Kwami L.G.A., in the south by Akko L.G.A. and east by Yamaltu

Deba L.G.A. Tashan Dukku livestock market is the largest livestock market in the metropolis. The markets serve the surrounding towns of Kwami, Kumo, Dadin-kowa and Akkoyel in the north, south, east and west respectively (Hamidu, 2014).

Numerous banks, filling stations and hotels exist in| Gombe metropolis to support the commercial activities. Another factor that led to the growth of the town is rural - urban migration experienced from the surrounding towns and villages. Moreover, the town has become a center of learning with numerous tertiary and secondary institutions established in the metropolis (Dauda and Lawali, 2014). Apart from the commercial activities in Gombe metropolis, most of the inhabitants engaged in agricultural activities. Gombe is one of the major food baskets in Nigeria; most of its landmass is cultivable and about 60% of the population engaged in agriculture. A number of cash crops and livestock are produced in Gombe. These are basic raw materials that can support various agro allied industries (Dauda and Lawali, 2014).

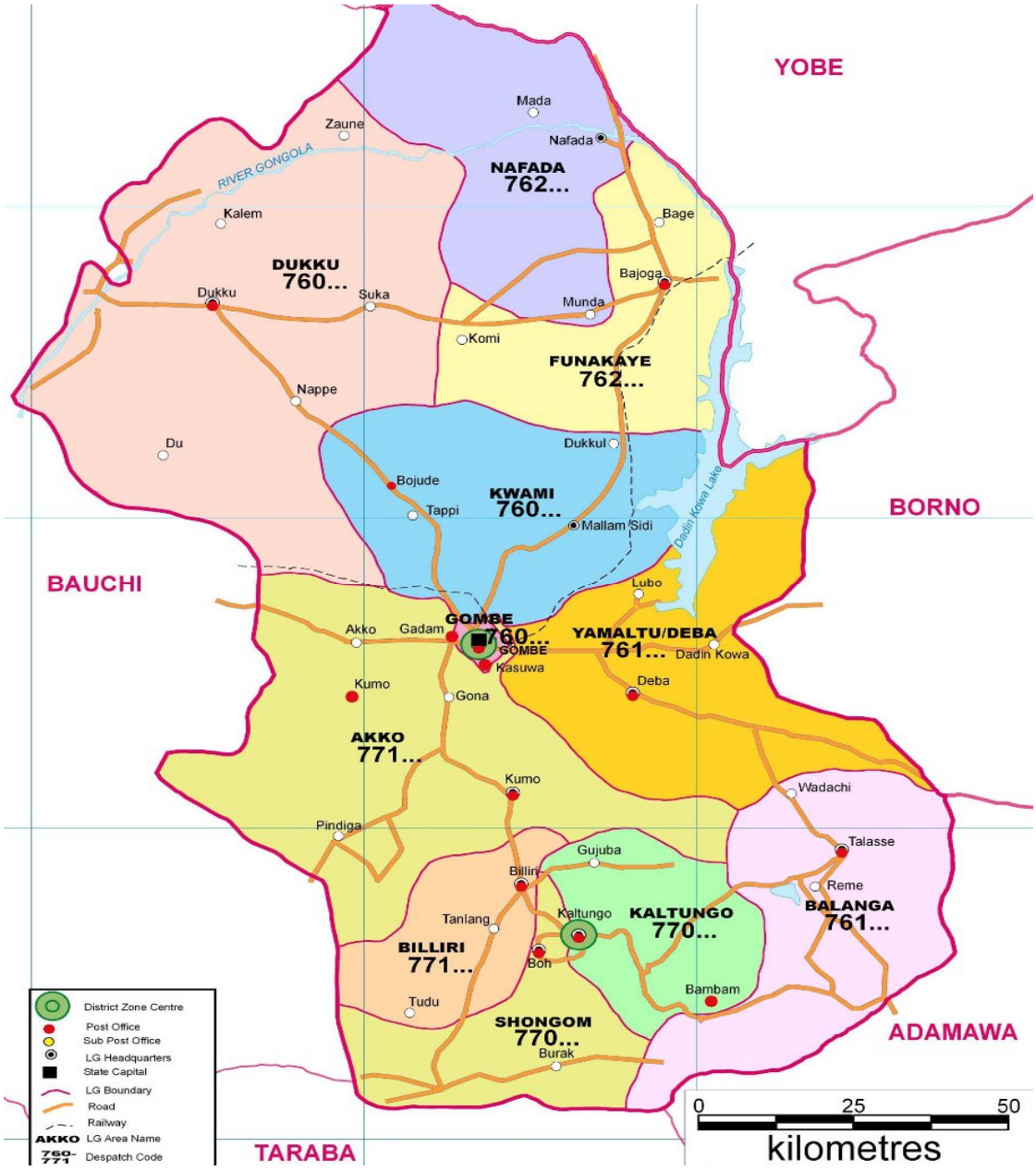

Figure 3.1: Map of the study area

3.2 Source of Sample

Fermented and fresh milk samples were collected for this study and the samples were collected from three different nono markets within Gombe metropolis namely: Gombe main market Tashan Dukku market and Tashan Shongo market and only fresh milk samples were collected from six herds at the time of milking.

3.3 Field Observation of the Environment and Milking Animals

Observation of the environmental sanitation of the site, type of water used, hygiene of the vendors, cleanliness of the personnel, the health of the animals as well as the sanitary conditions of the utensils used during milking and also the milking procedures were undertaken. (FAO, 2010)

3.4 Sample Size

Sixty samples were collected from each of the markets stated; thirty fresh and thirty fermented milk samples which give a total of one hundred and eighty samples (180). Fifteen fresh milk samples were collected from each herd.

3.5 Sample Collection

The samples were collected using systematic random sampling as described by Patton (1990). The samples were transported to the laboratory in sterile corked plastic tubes packed in an iced container. A total of one hundred and eighty fresh and fermented milk samples (90 samples each) were collected and analyzed for the presence of *Mycobacterium bovis*.

3.6 Determination of pH of the Milk Samples

About 10 mL of the milk sample was measured and dispensed into conical flask and its pH was determined using the pH meter (HI 3220 pH/ORP meter). The pH meter was standardized using standard buffer of pH 4.0 and 7.0 (AOAC, 1990 and FAO, 2010).

3.7 Determination of Temperature of the Milk Samples

The temperature of the fresh and fermented milk samples collected was determined at the time of collecting the samples and the temperature was measured by using thermometer immediately after collecting the samples in the sampling locations (Teshome *et al.,* 2015).

3.8 Determination of Turbidity of the Milk Samples

The turbidity of the samples was determined according to the FAO (2010). In this method, 20mL of the sample was measured and poured into a 50mL conical flask containing 4.0g of ammonium sulphate (NH_4SO_4). The mixture was shaken for 1minute to dissolve the ammonium sulphate (NH_4SO_4) and the mixture was then allowed to stand 5minutes and then filtered into a 100mL conical flask through filter paper. Thereafter, 5mL of the clear filtrate was then transferred into a beaker of boiling water for 5minutes. It was then cooled in cold water. The turbidity was then measured at 500nm by using a UV/ Visible spectrophotometer (GENWAY 63100).

3.9 Determination of the Total Titratable Acidity of the Milk Samples

Using the method described by (AOAC, 1990 and FAO, 2010) in this method about 10mL of the sample was measured and dispensed into a conical flask, 3 drops of phenolphthalein was then added. Thereafter, 0.1N NaOH was used to titrate to a noticeable pink colour for end point determination. The acidity will be calculated as lactic acid using the relationship:

$$\% \text{ Lactic Acid} = \frac{[\text{Titre value}] \times [0.1\text{N NaOH}] \times [\text{Equivalent Factor}]}{\text{Volume of Sample}} \times 100$$

Normality of NaOH = 0.1

Volume of Sample = 10ml

Equivalent factor = 0.009. This is because 0.009 gram of lactic acid is found in 1mL of 0.1N lactate.

Calculation for the preparation of 0.1N NaOH

Normality = Equivalent x concentration. But, Equivalent = $\frac{\text{Molar mass}}{\text{Valency}}$

Therefore, Normality = $\frac{\text{Molar mass}}{\text{Valency}}$ x concentration

Molar mass of NaOH is 40
The valency of NaOH is 1

Hence, for 0.1N NaOH, the normality is:

Normality = $0.1 \times \frac{40}{1}$

$= 0.1 \times 40$

$= 4g$

Therefore, 4g of NaOH was measured and dissolved in 1 liter of distilled water to obtain a concentration of 0.1N of NaOH.

3.10 Enumeration of the Total Aerobic Mesophilic Bacteria in the Milk Samples

Total aerobic mesophlic bacterial count was determined by using a pour plate method as described by FAO (2010). In this method one milliliter (1mL) of inoculum from 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions were transferred into duplicate petri dishes which were labeled accordingly. This was followed by pouring aseptically about 20-25mL of molten nutrient agar and was mixed by swirling the plates and later allowed to solidify. The plates were then incubated at 37^0 C for 24 hours. After incubation, plates containing 30-300 colonies were selected and the colonies were counted and recorded. Preliminary work was carried out to determine the concentrations that will give colonies ranges from 30 – 300. Only the dilutions that gives a colony ranges from 30 – 300 were transferred into duplicates of petri dishes for the remaining samples. The average was taking and the number obtained was multiplied by inverse

of the dilution factor. This gives the number of colony forming units per mL of each sample (CFU/mL).

3.11 Enumeration of the Anaerobic Mesophilic Bacteria in the Milk Samples

Anaerobic bacteria count was conducted using pour plate method. In this method, the samples were serially diluted and then 1mL of the inoculum from 10^{-2} and 10^{-3} dilutions were transferred into duplicate petri dishes which were labeled accordingly. This was followed by pouring aseptically 20mL of molten nutrients agar and it was mixed by swirling the plates and then it was allowed to solidify. The plates were then incubated anaerobically at 37^0 C for 24hours. After incubation, the colonies were counted and recorded, the average was taken and the number obtained was multiplied by the inverse of the dilution factor to obtain the number of colony forming units per mL of each sample, FAO (2010).

3.12 Detection of *Mycobacterium bovis* in the Milk Samples

3.12.1 Preparation of Lowenstein – Jensen medium

According to the laboratory services in tuberculosis control part III by World Health Organization (1998), about 37.2g of the Lowenstein – Jensen medium was weighed and dissolved in 600mL of distilled water; the media was shaken very well to dissolve the powder. After which 7.2g of sodium pyruvate was measured and added to the medium with continuous shaking until the medium become completely homogenized. The medium was then sterilised by autoclaving at 121^0C for 15minutes. It was then removed and allowed to cool.

Fresh eggs were then carefully cleansed with soap and water before it was soaked in 70% ethanol for 15minutes. The eggs were then removed and allowed to dry and then emptied inside a sterile blender. The egg was blended and allowed to settle before filtering through sterile cotton gauze. The filtered egg was then mixed with the media gently (to avoid bubbles) to homogeneity. The

medium was then dispensed in 6 - 8mL volumes into sterile McCartney bottles, the tubes were placed on the racks so as to achieve appropriate slope and they were inspissates at 80^0C for 45minutes. The bottles were then cooled and labeled to identify the batch and the date of preparation. The tubes were then kept in an upright position in the refrigerator (WHO, 1998).

3.12.2 Isolation of *Mycobacterium bovis*

For the isolation of *Mycobacterium bovis*, a modified method of Ben Kahla *et al.,* (2011), was used. In this method about 15mL of the milk sample was measured and decontaminated using 4% NaOH, and then neutralized with sterile normal saline. The suspension was then centrifuged at 3000rpm for 30minutes and the deposits were inoculated onto the already prepared Lowenstein – Jensen medium and incubated at 37^0C for 8 weeks. *M. bovis* characterization was performed by culture characteristics, Ziehl – Neelsen staining and the culture was confirmed using SD- Bioline test.

3.12.3 Decontamination of the Sample

The samples need to be prepared before processing for isolation of *Mycobacterium bovis*. The samples were decontaminated by using sodium hydroxide and concentrated by using sterile saline (modified petroff) method as described by world health organization (WHO, 1998).

3.12.4 Sodium Hydroxide (Modified Petroff) Method

This method is used widely in developing countries because of its relative simplicity and the fact that the reagents are easy to obtain. NaOH is toxic for both contaminants and tubercle bacilli; therefore, strict adherence to the indicated timing is required (WHO, 1998).

Reagents

1. 4% Sodium hydroxide (NaOH) solution:

- Sodium hydroxide pellets (analytical grade) = 4g

- Distilled water = 100mL

Procedure

About 4g sodium hydroxide pellets was weighed and dissolved in 100mL distilled water and the solution was sterilised by autoclaving at 121^0C for 15 minutes.

2. Sterile Saline

- Sodium chloride pellets (analytical grade) = 0.85g

- Distilled water = 100mL

Procedure

About 0.85g of sodium chloride pellet was weighed and it was dissolved in 100mL of distilled water. The solution was sterilized by autoclaving at 121^0C for 15 minutes (WHO, 1998).

3.12.5 Procedure for Decontamination

Fifteen ml (15mL) of the sample was put into a falcon tubes and equal amount of 4% sodium hydroxide i. e. 15mL of 4% NaOH was added to the sample. The container was tightly closed and it was shaked to digest the sample. The container was then allowed to stand for 15minutes at room temperature with occasional shaking. The sample was then centrifuge at 3000 rpm for 15 minutes.

After centrifugation, the supernatant was decanted and the sediment remained in the container. Then about 15mL of sterile saline was added and the sample centrifuge further for another 15minutes at 3000 rpm. After centrifugation, the supernatant was decanted and then the sediment was inoculated onto a Lowenstein – Jensen culture media (WHO, 1998).

3.12.6 Inoculation on Lowenstein – Jensen Medium

After sample decontamination, using a sterile Pasteur pipette; the slope of Lowenstein – Jensen media was inoculated with 0.2 to 0.4mL (2 – 4 drops) of the centrifuged sediment and distributed over the surface (WHO, 1998).

3.12.7 Incubation of the Culture Medium

After inoculation of the sample onto the Lowenstein – Jensen media, all the culture tubes were incubated at 37^0C and they are observed for the presence of growth daily for seven days and then weekly for a period of eight (8) weeks. After eight weeks those with a growth a recorded as positive while those that shows no growth were discarded and recorded as negative culture (WHO, 1998).

3.13 Ziehl-Neelsen Staining

The Ziehl–Neelsen stain, also known as the acid-fast stain, widely used differential staining procedure. In this type some bacteria resist decolorization by both acid and alcohol and hence they are referred as acid fast organisms. This staining technique divides bacteria into two groups namely acid-fast and non acid-fast. This procedure is extensively used in the diagnosis of tuberculosis and leprosy (Delisle and Tomalty, 2002).

3.13.1 Ziehl–Neelsen Staining Procedure

A smear of was prepared and allowed to dry and then heat fixed. The smear was then flooded with Carbol Fuchsin stain and heat for 5 minutes and it was then rinsed with de- ionized water. Acid alcohol was then flooded on the slide for 15 seconds until the smear is sufficiently decolorized, i.e. pale pink. The slide was then rinsed with de - ionized water and Loeffler's Methylene Blue stain was added as counter stain. The Loeffler's Blue stain was allowed to stay on smear for 1 minute and it was rinsed with clean water and it was allowed to dry. The slide

was then examined microscopically using x10 oil immersion (Delisle and Tomalty, 2002; Cheesbrough, M. 2006).

3.14 SD-Bioline Test for the Confirmation of *Mycobacterium bovis*

SD–Bioline Rapid test was performed according to the manufacturer's guidelines. Three to four colonies of mycobacterial strains grown in Löwenstein-Jensen media were emulsified in 100µL of extraction buffer, then 50µL was placed in sample wells of the test and the results was visually assessed based on color development after incubation at room temperature for 15 min. The presence of two color bands within the result window indicates a positive result while presence of only control band within the result window indicates a negative result (WHO, 2006 and Hyeon-Seop *et al.*, 2015).

3.15 Enumeration of Coliform Bacteria in the Milk Samples

Coliform count was done using the "Most Probable Number (MPN) technique" also known as the Multiple Tube Fermentation Test (MTFT). In this method, the samples were serially diluted by transferring 1mL of the sample into 9mL of distilled water. The dilutions were 10-fold (10x), 100-fold (100x) and 1000-fold (1000x). Fifteen tubes were groups into fives to make three (3) set. 1mL from each of the dilutions was transferred into first set of 5 tubes containing 9mL of lactose broth and inverted Durham tubes; then 1mL was added to the second set of five tubes and then to the third set. The tubes were incubated at 37^0C for 24 – 48hrs. Presumptive positive tubes were recorded for any growth accompanied with gas production in the Durham's tube. Presumption positive tubes were confirmed by sub-culturing two loopful from any tube showing gas production into a tube of brilliant green lactose bile broth containing inverted Durham tubes. The tubes were then incubated at 37^0C for 24 – 48hrs; gas production in the Durham's tube confirmed the positive results and the number of confirmed positives tubes were recorded. The Most Probable Number was determined by comparing the number of positives tubes with MPN

index table APHA (1992). The standard coliform count not to exceed 10/mL, but the goal - not detectable (SMEDP, 16th/17th ed.).

3.16 Determination of Nutritional Composition of the Milk Samples

3.16.1 Proximate Analysis

3.16.1.1 Determination of Moisture Content of the Milk Sample

Moisture is determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. About 5mL of the sample was measured into a silica dish that was previously washed and dried and it was then weigh and recorded as W_1. The sample was then dried in an oven at 105^0C for 3hours. It was then allowed to cool in the desiccator and weight again and recorded as W_2 (AOAC, 1990).

The moisture content of the samples can be calculated by using the following relation or using the formula below:

$$\% \text{ Moisture} = \frac{W_1 - W2}{\text{Wt of Sample}} \times 100$$

Where W_1 = Weigh of the dish + samples before drying

W_2 = Weigh of the dish + samples after drying.

3.16.1.2 Determination of Protein Content of the Milk Sample

The proteins content was determined by using Biuret method. The biuret method is based on the reactions that occur between cupric ions in the reagents and the peptide bonds of the proteins molecules in alkaline solutions to form blue – violet or purple coloured complexes. The absorbance of the colour is measured using a calorimeter at 540nm (Ochei and Kolhatkar, 2000).

Reagents

1. Biuret reagents (stock solution):- This reagent was prepared by dissolving 45g of sodium potassium tartarate in 400mL of 0.2N NaOH. Therefore, to prepare 0.2N NaOH:

$$\text{Normality} = \text{Equivalent} \times \text{concentration}$$

$$\text{But equivalent} = \frac{\text{Molar mass}}{\text{Valency}}$$

Molar mass of NaOH = 40

Valency of NaOH = 1

$$\text{Hence, Normality} = \frac{\text{Molar mass}}{\text{Valency}} \times \text{concentration}$$

$$= \frac{40}{1} \times 0.2$$

$$= 8\text{g/L of NaOH}$$

Therefore, 8g/L of NaOH was weighed and dissolved in 1litre of distilled water to obtain 0.2N of NaOH. Then 45g of sodium potassium tartarate was measured using a weighing balance and it was dissolved in 400mL of 0.2N NaOH. After that, 15g of copper sulphate and 5g of potassium iodide was added to the solution and the volume was filled from 400mL up to 1 liter.

2. Biuret working solution: - this reagent was prepared by diluting 200mL of the stock solution to 1 liter with 0.2N NaOH containing 5g/l of potassium iodide.

3. Tartarate iodide solution:- this reagent was prepared by dissolving 9g of sodium potassium tartarate in 1litre of 0.2N NaOH containing 5g/l potassium iodide.

4. Standard protein of concentration 5g/l.

Method

Five different test tubes were set and label as Test, Test Blank, Standard, Standard Blank and Blank respectively. Then 5mL of the working biuret reagent was put into tubes label as Test, Standard and Blank. Also 5mL of the tartarate iodide solution was added into test tubes label as Test blank and Standard blank.

However, 0.05mL of the sample was added into the test tubes label as Test and Test blank, followed by 0.05mL of the Standard added to the Standard and Standard blank. Finally, 0.05mL of distilled water was added into the test tube label as Blank.

After that, the test tubes were mixed very well and then leave at room temperature for 10 minutes. After 10 minutes, the blank was used to zero the calorimeter and the absorbance of the test tubes was taken at 540nm. The amount of protein in the samples was calculated by using the following relation / formula:

$$\frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times \text{Concentration of the Standard}$$

Concentration of the standard = 5g/l

3.16.1.3 Determination of Fat Content of the Milk Sample

About 5mL of the sample was measured and dispensed into a small flask and incinerated to ash at 550^0C for 3hours in the furnance. After dying, a glass rod was used to scratch the ash from the bottom of the flask and then 10mL of petroleum ether was added and it was shaken for about 15minutes. All the contents of the flask were then transferred to a pre – weighed flask and the flask was put in oven to evaporate the solvent. After the solvent was evaporated, the flask was removed and allowed to cool and reweighed (AOAC, 1990). The amount of fat was calculated using the formula below:

$$\% \text{ Fat} = \frac{(\text{wt of dish + extract}) - \text{wt of the flask}}{\text{Wt of samples}} \times 100$$

3.16.1.4 Determination of Ash Content of the Milk Sample

About 5mL of the sample was measured into a pre – weighed crucible. The crucible containing the sample was then transferred into an oven and the sample was dried at 105^0C for 3hours. After the sample was dried, the crucible was then transferred into a furnance using a pair of tongs and the oven was ignited for 3hours at 550^0C. After 3hours, the crucible was then removed using a pair of tongs and it was allowed to cool in the desiccator and weighed immediately (AOAC, 1990). The amount or percentage of ash present in the samples was calculated using the following formula:

$$\% \text{ Ash} = \frac{(\text{wt of crucible + ash}) - \text{wt of crucible}}{\text{Wt of sample}} \times 100$$

3.16.1.5 Determination of the Carbohydrate of the Milk Sample

According to (AOAC, 1990), the carbohydrate content of the milk sample was determined by subtracting from 100 the sum of the percentage moisture, ash, protein and fat. The remainder value gives the carbohydrate content of the milk sample. The formula can be written as:

% Carbohydrate = 100 – (sum of moisture, protein, ash and fat)

3.16.2 Analysis of Mineral Elements (Mg, Mn, Ca, K, Na) in the Samples

The above mineral content was determined by using a modified method of Lawal and Adedeji (2013). Two milliliter (2mL) of each sample was incinerated to a white ash at 550°C in a muffle furnace for 3 hours, it was then be allowed to cool and the ash was washed into 250mL beaker with de - ionized water and it was then dissolved with a drop of HCL and then filtered with whatman no 1 filter paper. The volume was made up to 100mL with de - ionized water. The

minerals elements were determined using spectrometry method of Atomic Absorption Spectrophotometer (Buck 205) by Buck Scientific. Samples were aspirated and the mean signal response was recorded at each of the element respective wavelength (Lawal and Adedeji, 2013).

3.16.3 Analysis of Vitamins Content of the Milk Sample

The vitamins contents of the milk samples were determined using the official methods of the Association of Official Analytical Chemists (AOAC, 1990). The amount of vitamins such as vitamin A, vitamin C and vitamin E present in the samples were analyzed by using UV/Visible spectrophotometer (GENWAY 63100).

3.16.3.1 Determination of Vitamin A (Retinol) in the Milk Sample

For the determination of vitamin A, 2ml of the milk sample was measured and dissolved in 20mL of n – hexane in test tubes for 10minutes. Then 3mL of the upper n – hexane extract was transferred into a dry test tube in duplicates and evaporated to dryness. Then 0.2mL of acetic anhydride chloroform reagent was added and then 2mL of 50% trichloroacetic acid (TCA) was then added and then the absorbance was taken at 620nm (AOAC, 1990).

After the analysis, the vitamin A concentration in the milk samples was calculated by using the following relationship:

$$\text{Vitamin A} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times \text{Concentration of Standard}$$

Standard absorbance = 0.027

Concentration of standard = 10 IU

3.16.3.2 Determination of Vitamin C (Ascorbic Acid) in the Milk Sample

For vitamin C, 2mL of the milk sample was measured and dissolved in 10mL of 0.4% oxalic acid in a test tube for 10minutes. It was then centrifuge for 15minutes and the solution was

filtered. Then 1mL of the filtrate was transferred into a dry test tube in duplicates and then 9mL of 2,6 – dichlorophenol indophenol was added and the absorbance was taken at 520nm (AOAC, 1990).

After the analysis, the vitamin C concentration of the milk samples was calculated by using the following relationship:

$$\text{Vitamin C} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times \text{Concentration of Standard}$$

Standard absorbance = 0.471`

Concentration of standard = 1mg/mL

3.16.3.3 Determination of Vitamin E (Tocopherol) in the Milk Sample

About 2mL of the sample was measured and dissolved in 20mL of n – hexane in a test tube and stand for 10minutes. The sample was then centrifuge for 10minutes. The solution was then filtered and then 3mL of the filtrate was transferred into a dry test tube in duplicate and evaporated to dryness. Then 2mL of 0.5N alcoholic potassium hydroxide was added and boiled for 30minutes in a water bath. Then 3mL of n – hexane was added and was shaken vigorously. The n – hexane was then transferred into another set of test tubes and evaporated to dryness.

After that, then 2mL of ethanol was added to the residue and then 1mL of 0.2% ferric chloride; then 1ml of 0.5% α^1, α^1 – dipyridyl was added followed by 1mL of ethanol to make it up to 5mL. Then the absorbance was taken at 520nm (AOAC, 1990).

Calculations:

$$\text{Vitamin E} = \frac{\text{Test Absorbance}}{\text{Standard Absorbance}} \times \text{Concentration of Standard}$$

Standard absorbance = 0.291

Concentration of standard = 10 IU

3.17 Statistical Analysis of the Data

Analysis of variance protocol was used to confirm the significance level of the differences at ($P<0.05$) in the mean values of the physiochemical parameters as well as the significance difference between fresh and fermented milk samples collected at different location in the study area.

CHAPTER FOUR

4.0 RESULTS

Milk was observed to be a commodity available in the study area. It was obtained from cows mainly by local milking farmers. Women are the major processors and marketers of both fresh and fermented form of the milk. It was observed that milking was done only once in the day, mostly early in the morning. It was also observed that the pastoralist were not cleaning the floor before milking and they were not washing the whole animals but they only washed the udder and the teats of the animals before they start milking the animal. All the pastoralist practiced the same way, they washed the udder and the teat before milking but they don't use clean clothes to dry the udder after washing. In the herd, the manure is not removed daily and the farmers were not using sterile hand gloves during milking. Moreover, flies were observed to be presence in the environment were milking of the cows take place (Table 1). Also in the market the vendors are not staying in a hygienic environment and they don't always close their calabash while selling the milk and they hold the cup they used to measure the milk from every angle. The milk was

observed to have its white colour and its natural smell; it is consistent, turbid and creamy with little viscosity (Table 2). The water used by the personnel during milking and other processes is not clean. Also in the market the vendors are not staying in a hygienic environment and they don't always close their calabash while selling the milk and they hold the cup they used in measuring the milk from every angle.

Table 1: General Practices Observed in all Farms in Gombe Metropolis

Activities	Existing condition
Milking frequency/day	93%
Cleaning the land for animals	7%
Clean water	20%
Washing udder and teats	83%
Used clean cloth to dry udder after washed	17%
Use of disposable gloves	0%
Manure removal	47%
Presence of flies in the environment	'67%
Discarded first milk flow	93%
Used clean and dry milk container	53%

Table 2: Range of Some Physical Features of Milk Samples from Gombe Metropolis.

Nature of the samples	Fresh	Fermented
pH	6.69±0.08 – 6.7±0.09	4.19±0.3 – 4.59±0.2
Temperature	20.45±1.3 – 20.97±2.0	9.86±1.8 – 10.51±1.5
Turbidity	5.70±2.02 – 16.57±3.56	1.90±1.47 - 12.1±2.75
Viscosity	Slightly Viscous	Highly viscous
Texture ‘‘	Creamy	Creamy

4.1 Mean pH and Temperature of the Milk Samples Obtained from Different Markets within Gombe Metropolis

The results of the pH as described in Table 3 ranges from slightly acidic as in fresh milk to acidic as in fermented milk. The results showed that fresh milk was in the range of 6.69±0.08 to 6.70±0.09 while the fermented milk ranges from 4.19±0.03 to 4.59±0.27. The results in Table 4 indicated that fermented milk samples collected from Tashan Shongo market has the highest temperature with 10.51±1.5^{0}C; while samples collected from Tashan Dukku market has the lowest temperature obtained with 9.86±1.8^{0}C. The results also showed that the average of fresh milk samples is significantly higher with 21.35±1.9^{0}C than that of the fermented milk samples with the average mean of 10.26±1.7^{0}C.

4.2 Mean Turbidity and Total Titratable Acidity of the Milk Samples Obtained from Different Markets within Gombe Metropolis

Table 5 showed the turbidity value of 1.90±1.47NTU to 12.07±2.75NTU of fermented milk from Tashan Shongo market. The turbidity in fresh milk ranges from 5.70±2.02 NTU to 16.57±3.56 NTU. Table 6 explained the total titratable acidity of the samples; the results showed that Gombe

main market has the highest value of total titratable acidity in fermented milk samples with 0.18±0.04 and Tashan Shongo market having the lowest value in fermented milk samples with (0.15±0.03). Fresh milk samples collected from Tashan Dukku market has the peak of acidity 0.17±0.03 and those collected from Gombe main market has the lowest with 0.12±0.02.

4.3 Mean Aerobic and Anaerobic Mesophilic Bacterial Count of the Milk Samples Obtained from Different Markets within Gombe Metropolis

The results also indicated a range of aerobic mesophilic plate count among fermented milk as $6.53x10^{8}$CFU/mL to $9.18x10^{8}$CFU/mL and in fresh milk it is between $5.1x10^{8}$CFU/mL to $8.73x10^{8}$CFU/mL. Also the anaerobic bacteria count ranges from $3.60x10^{3}$CFU/mL to $1.21x10^{3}$CFU/mL in fermented milk samples and $2.65x10^{3}$CFU/mL to $2.33x10^{3}$CFU/mL in fresh milk samples Table 7.

4.4 Occurrence of Mycobacterium bovis and Mean Coliform Count in Fresh and Fermented Milk in Gombe Metropolis

Mycobacterium bovis isolated from both fresh and fermented milk ranges from 6(6.67%) in fermented milk to 21(23.33%) in fresh milk (Table 8). The coliform count in fresh milk is higher than in fermented milk samples, the coliform count ranges from 4.93±2.3MPN/100mL to 10.07±4.9MPN/100mL and 5.97±2.1MPN/100mL to 6.17±2.4MPN/100mL in fresh and fermented milk respectively (Table 9). The overall mean 7.22±4.6MPN/100mL of fresh milk samples is higher than 6.10±2.2MPN/100mL of fermented milk samples.

Table 3: Mean pH of the Milk Samples Obtained from Herds and Market in Gombe

Fermentation Status	Location	Number of samples	pH
Fermented	GMM	30	4.59±0.27
	TDM	30	4.47±0.26
	TSM	30	4.19±0.34
	Total	90	4.42±0.33
Fresh	GMM	30	6.69±0.11
	TDM	30	6.69±0.08
	TSM	30	6.70±0.09
	Total	90	6.69±0.89

Standard acceptable pH of Milk: 6.7 for fresh milk and 4.3 for fermented milk (FAO, 2010)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

$P < 0.05$

Mean = 5.56±1.17

S.E = 0.16

S.D = 1.17

Table 4: Mean Temperature of the Milk Samples; it is Measures in Degree Celsius

Location	Fermentation Status	Number of samples	Temperature (^{0}C)
GMM	Fermented	30	10.43±1.8
	Fresh	30	20.45±1.3
	Total	60	15.16±5.6
TDM	Fermented	30	9.86±1.8
	Fresh	30	20.97±2.0
	Total	60	15.69±5.6
TSM	Fermented	30	10.51±1.5
	Fresh	30	22.62±1.7
	Total	60	16.56±6.3

Standard Milk Temperature: It ranges from 7 – 10^{0}C (FAO, 2010)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

$P < 0.05$

Mean = 15.80±5.8

S.E = 0.126

S.D = 5.8

Table 5: Mean Turbidity of the Milk Samples in Nephlometric Turbidity Unit (NTU).

Fermentation Status	Location	Number of samples	Turbidity
Fermented	GMM	30	4.70±2.54
	TDM	30	1.90±1.47
	TSM	30	12.07±2.75
	Total	90	6.22±4.89
Fresh	GMM	30	9.77±2.53
	TDM	30	5.70±2.02
	TSM	30	16.57±3.56
	Total	90	10.68±5.28

Standard turbidity of milk: Zero (0) (FAO, 2010)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

$P < 0.05$

Mean = 8.45±5.54

S.E = 0.191

S.D = 5.54

Table 6: Mean Total Titratable Acidity of the Milk Samples from Gombe Metropolis

Fermentation Status	Location	Number of samples	Total titratable acidity
Fermented	GMM	30	0.18±0.04
	TDM	30	0.16±0.04
	TSM	30	0.15±0.03
	Total	90	0.16±0.04
Fresh	GMM	30	0.12±0.02
	TDM	30	0.17±0.03
	TSM	30	0.16±0.02
	Total	90	0.15±0.03

Standard Acceptable acidity: 0.14 - 0.16% (AOAC, 1990)

Key:	$P < 0.05$
Gombe Main Market (GMM)	Mean = 0.16±0.04
Tashan Dukku Market (TDM)	S.E = 0.002
Tashan Shongo Market (TSM)	S.D = 0.04

Table 7: Total Aerobic and Anaerobic Mesophilic Bacterial Count of the Milk Samples. The Result is Expresses in Number of Colony Forming Unit per mL (CFU/mL).

Location	Fermentation Status	Number of samples	Aerobic Bacteria count	Anaerobic Bacteria count
GMM	Fermented	30	$9.18x10^8$	$1.21x10^3$
	Fresh	30	$8.73x10^8$	$2.65x10^3$
	Total	60	$1.79x10^9$	$1.93x10^3$
TDM	Fermented	30	$6.57x10^8$	$1.34x10^3$
	Fresh	30	$5.18x10^8$	$2.33x10^3$
	Total	60	$1.18x10^9$	$1.84x10^3$
TSM	Fermented	30	$6.53x10^8$	$3.60x10^3$
	Fresh	30	$6.06x10^8$	$2.64x10^3$
	Total	60	$1.26x10^9$	$3.12x10^3$
Total	Fermented	90	$2.23x10^9$	$6.15x10^3$
	Fresh	90	$1.99x10^9$	$7.62x10^3$
	Total	180	$4.22x10^9$	$1.38x10^4$

Standard acceptable value = $2.0x10^4$ and $1.0x10^5$ CFU/mL for pasteurized and non-pasteurized milk respectively. FDA (2007)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

$r = - 0.36$

Table 8: Occurrence of *Mycobacterium bovis* from Fresh and Fermented Milk Samples

Location	Fresh				Fermented			
	No of Samples	LJ	ZN	SD Bioline	No of Samples	LJ	ZN	SD Bioline
GMM	30	10(33.33%)	10(33.33%)	8(26.67%)	30	8(26.67%)	3(10%)	2(6.67%)
TDM	30	7 (23.33%)	7 (23.33%)	7(23.33%)	30	9(30%)	2(6.67%)	2(6.67%)
TSM	30	7 (23.33%)	7 (23.33%)	6(20%)	30	7(23.33%)	2(6.67%)	2(6.67%)
Total	90			21(23.33%)	90			6(6.67%)

Key:

LJ = Lowenstein Jensen

ZN = Ziehl Neelsen

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

Table 9: Total Coliform Count of the Milk Samples Expressed in MPN/100mL.

Location	Fermentation Status	Number of samples	Total Coliform Count (MPN/100mL)
GMM	Fermented	30	6.17±2.4
	Fresh	30	6.67±4.5
	Total	60	6.42±3.6
TDM	Fermented	30	6.17±2.1
	Fresh	30	4.93±2.3
	Total	60	5.55±2.2
TSM	Fermented	30	5.97±2.1
	Fresh	30	10.07±4.9
	Total	60	8.02±4.3

Standard acceptable value: Not exceed 10 MPN/100mL APHA (1992)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

$P < 0.05$

Mean = 6.66±3.6

S.E = 0.244

S.D = 3.6

4.5 Mean Moisture and Protein of the Milk Samples Obtained from Different Markets Within Gombe Metropolis

Fermented milk has high moisture content than fresh milk. In fermented milk, the moisture ranges from 8.52±3.4mg/100mL to 51.53±12.0mg/100mL and the fresh milk samples ranges from 0.86±0.2mg/100mL to 1.24±0.2mg/100mL. The protein contents in fermented milk ranges from 1.58±0.9mg/100mL to 20.89±20.1mg/100mL. The fresh milk has the highest protein contents of 52.50±9.9mg/100mL and lowest value of 49.58±13.9mg/100mL. In both fresh and fermented milk, samples collected from Tashan Dukku market have the lowest protein contents (Table 10).

4.6 Mean Fat, Ash and Carbohydrate of the Milk Samples Obtained from Different Markets within Gombe Metropolis

Fat content is highest in fermented milk samples collected from Tashan Shongo market with 5.73±0.6mg/100mL and lowest in samples from Gombe main market with 0.57±0.4mg/100mL. In fresh milk, the fat content is highest in Tashan Shongo market with 9.67±0.9mg/100mL and lowest in Gombe main market with 9.27±1.9mg/100mL. The ash contents in fermented milk range from 2.23±2.6mg/100mL to 2.94±2.6mg/100mL while in fresh milk samples it ranges from 2.36±0.5mg/100mL to 2.52±0.5mg/100mL. The carbohydrate contents in fermented milk ranges from 40.76±14.2mg/100mL to 76.88±9.7mg/100mL but in fresh milk it ranges from 34.60±10.4mg/100mL to 36.10±10.4mg/100mL (Table 11).

4.7 Mean Magnesium and Manganese of the Milk Samples Obtained from Different Markets within Gombe Metropolis

Magnesium and manganese concentration in the samples are expressed mg/100mL. The result showed magnesium concentration of 23.53±21.9mg/100mL in fermented milk at Tashan Shongo market. In samples collected from Gombe main market it was 15.00±20.0mg/100mL. The concentration of magnesium in fresh milk ranges from 15.07±2.0mg/100mL to 15.63±2.1mg/100mL. Manganese concentration of fermented milk is highest in samples collected from Tashan Shongo market with 23.13±20.6mg/100mL and lowest in samples collected from Gombe main market with 16.37±20.4mg/100mL (Table 12).

4.8 Mean Calcium, Potassium and Sodium of the Milk Samples Obtained from Different Markets within Gombe Metropolis

Table 13 showed the concentration of calcium, potassium and sodium in mg/100mL. Calcium concentration ranging from 217.87±84.9mg/100mL to 251.65±79.1mg/100mL in fermented milk samples, while in fresh milk samples it ranges from 267.47±21.9mg/100mL to 274.28±23.8mg/100mL.The result of potassium concentration showed that fresh milk samples has concentration ranging from 136.35±15.4mg/100mL to 137.67±13.4mg/100mL while fermented milk samples ranges from 181.47±15.5mg/100mL to 183.65±16.8mg/100mL. However, concentration of sodium of 316.83±196.3mg/100mL was found in Tashan Shongo market and those collected from Gombe main market showed 307.97±202.1mg/100mL. The fresh milk showed that samples collected from Tashan Shongo market has concentration of 184.00±184.3mg/100mL while samples collected from Gombe main market has mean of sodium with 153.50±165.7mg/100mL. Both fresh and fermented milk samples from Tashan Shongo market showed high concentration of sodium ion but lowest in samples collected from Gombe main market in both fresh and fermented milk samples analysed.

4.9 Mean Vitamins (A, C and E) of the Milk Samples Obtained from Different Markets Within Gombe Metropolis

The results of the analysis in Table 14 showed the values of vitamin A, C and E content in both fresh and fermented milk samples. The values of vitamin A ranges from 47.48±22.1 IU/100mL to 75.23±25.2 IU/100mL and 156.23±19.7 IU/100mL to 179.78±26.8 IU/100mL in fermented and fresh milk respectively. Vitamin C content of the samples ranges from 0.59±0.1mg/100mL to 0.76±0.2mg/100mL and 1.59±0.3mg/100mL to 1.85±0.4mg/100mL in fermented and fresh milk samples respectively. The result also showed that both fresh and fermented milk samples collected from Tashan Shongo market have the lowest concentration of vitamin C. The results also revealed that the concentration of vitamin E ranges from 0.58±0.2mg/100mL to 0.77±0.3mg/100mL and 0.32±0.2mg/100mL to 0.34±0.20mg/100mL in fermented and fresh milk samples respectively.

Table 10: Moisture and Protein Content of the Milk Samples Expressed in mg/100mL.

Location	Fermentation Status	Number of samples	Moisture	Protein
GMM	Fermented	30	51.53±12.0	4.28±3.8
	Fresh	30	1.11±0.2	52.50±9.9
	Total	60	26.32±26.8	28.39±25.4
TDM	Fermented	30	15.50±8.9	1.58±0.9
	Fresh	30	0.86±0.2	49.58±13.9
	Total	60	8.18±9.7	25.58±26.1
TSM	Fermented	30	8.52±3.4	20.89±20.1
	Fresh	30	1.24±0.2	52.06±9.5
	Total	60	4.88±4.39	36.48±22.1
Total	Fermented	90	25.18±20.9	8.92±14.5
	Fresh	90	1.07±0.2	51.38±11.2
	Total	180	13.12±19.1	30.15±24.9

Standard Acceptable limit:
Moisture in Milk: It should not be less than 87.3mg/100mL
Protein in the milk: It should not be less than 2.9mg/100mL
Source: FSA (2002)

Key: r = - 0.75

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

Table 11: Fat, Ash and Carbohydrate Content of the Milk Samples Expressed in mg/100mL.

Location	Fermentation Status	Number of samples	Fat	Ash	Carbohydrate
GMM	Fermented	30	0.57±0.4	2.94±2.6	40.76±14.2
	Fresh	30	9.27±1.9	2.52±0.5	34.60±10.4
	Total	60	4.92±4.6	2.73±1.9	37.68±12.7
TDM	Fermented	30	3.73±1.3	2.31±2.8	76.88±9.7
	Fresh	30	9.48±1.4	2.51±0.5	36.10±10.4
	Total	60	6.61±3.2	2.41±1.9	56.49±22.8
TSM	Fermented	30	5.73±0.6	2.23±2.6	62.54±21.3
	Fresh	30	9.67±0.9	2.36±0.5	34.67±9.4
	Total	60	7.69±2.1	2.29±1.8	48.61±21.5
Total	Fermented	90	3.34±2.3	2.49±2.6	60.06±21.6
	Fresh	90	9.47±1.4	2.46±0.5	35.12±9.9
	Total	180	6.41±3.6	2.48±1.9	47.59±20.9

Standard acceptable value:
Fat: It should not be less than 4.0mg/100mL
Ash: It should not be less than 0.7mg/100mL
Carbohydrate: It should not be less than 4.7mg/100mL
Source: FSA (2002)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

Statistical Inference

$P < 0.05$

$F_{cal.} = 0.043$

$F_{Critical} = 0.19$

Table 12: Concentration of Magnesium and Manganese in the Milk Samples Expressed in mg/100mL

Location	Fermentation Status	Number of samples	Magnesium	Manganese
GMM	Fermented	30	15.00±20.1	16.37±20.4
	Fresh	30	15.53±2.0	0.07±0.03
	Total	60	15.27±14.1	8.22±16.5
TDM	Fermented	30	17.63±20.5	17.67±20.6
	Fresh	30	15.63±2.1	0.08±0.03
	Total	60	16.63±14.5	8.87±16.9
TSM	Fermented	30	23.53±20.9	23.13±20.6
	Fresh	30	15.07±2.0	0.07±0.02
	Total	60	19.30±15.4	11.60±18.5
Total	Fermented	90	18.72±20.6	19.06±20.5
	Fresh	90	15.41±2.0	0.07±0.03
	Total	180	17.07±14.7	9.56±17.3

Standard acceptable value:
Magnesium: Should not be less than 11mg/100mL
Manganese: Trace
Source: FSA (2002)

Key: r = + 0.75

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

Table 13: Concentration of Calcium, Potassium and Sodium in the Milk Samples Expressed in mg/100mL.

Location	Fermentation Status	Number of samples	Calcium	Potassium	Sodium
GMM	Fermented	30	217.87±84.9	181.47±15.5	307.97±202.1
	Fresh	30	274.28±23.8	137.67±13.4	153.50±165.7
	Total	60	246.07±68.1	159.57±26.4	230.73±199.1
TDM	Fermented	30	225.25±86.8	183.65±16.8	316.67±196.4
	Fresh	30	267.47±21.9	136.35±15.4	183.17±188.1
	Total	60	246.36±66.3	160.00±28.7	249.92±202.2
TSM	Fermented	30	251.65±79.1	183.65±16.8	316.83±196.3
	Fresh	30	269.72±21.4	136.35±15.4	184.00±184.3
	Total	60	266.80±58.1	160.00±28.7	250.42±200.3
Total	Fermented	90	231.59±83.9	182.92±16.2	313.82±196.1
	Fresh	90	270.49±22.3	136.79±14.6	173.56±178.2
	Total	180	251.40±64.3	159.86±27.8	243.69±199.6

Standard acceptable value:
Calcium: It should not be less than 112mg/100mL.
Potassium: It should not be less than 160mg/100mL.
Sodium: It should not be less than 4.4mg/100mL.
Source: FSA (2002)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

Statistical Inference

Location: $P > 0.05$

Fermentation Status: $P < 0.05$

$F_{Cal.} = 0.097$

$F_{Critical} = 0.198$

Table 14: Vitamin A, C and E Concentration of the Milk Samples Obtained from Herds and Market in Gombe Metropolis

Location	Fermentation Status	Number of samples	Vitamin A IU/100mL	Vitamin C mg/100mL	Vitamin E mg/100mL
GMM	Fermented	30	58.58±21.2	0.76±0.2	0.58±0.21
	Fresh	30	156.23±19.7	1.69±0.3	0.32±0.20
	Total	60	107.41±53.3	1.22±0.5	0.45±0.24
TDM	Fermented	30	75.23±25.2	0.72±0.2	0.77±0.33
	Fresh	30	179.78±26.8	1.85±0.4	0.34±0.20
	Total	60	127.51±58.7	1.29±0.6	0.55±0.35
TSM	Fermented	30	47.48±22.1	0.59±0.1	0.62±0.24
	Fresh	30	172.33±20.3	1.59±0.3	0.33±0.21
	Total	60	109.91±66.4	1.09±0.6	0.48±0.26
Total	Fermented	90	60.43±25.4	0.69±0.2	0.66±0.27
	Fresh	90	169.45±24.3	1.71±0.4	0.33±0.20
	Total	180	114.94±60.0	1.20±0.6	0.49±0.29

Standard acceptable value:
Vitamin A: It should not be less than 46 IU/100mL
Vitamin C: It should not be less than 2mg/100mL
Vitamin E: It should not be less than 0.08mg/100mL
Source: FSA (2002)

Key:

Gombe Main Market (GMM)

Tashan Dukku Market (TDM)

Tashan Shongo Market (TSM)

Statistical Inference

$P < 0.05$

$F_{Cal.} = 102625$

$F_{Critical} = 5.05$

CHAPTER FIVE

5.0 DISCUSSION

During the study, it was observed that not all the containers and utensils for milking and processing are clean and none of the pastoralist uses disposable gloves during milking (0%). Only 17% of the herds used clean clothes to dry the udder of the animals after washing and 93% of them discard the first milk flow. Presence of flies is 67% in the herds visited for sample collection. Almost all the herds milk their animals once in a day (93%). Most of them didn't clean the land for the animals daily; this may be attributed to the fact that the animals are large in number and they occupy a very large area of land. The milk samples were found to have standard physico-chemical parameters mostly within the internationally acceptable limit.

The pH of the fermented milk is significantly higher than those of the fresh milk. The average pH of the fermented milk is 4.4±0.21 while that of the fresh milk is 6.7±0.08. The result showed that all the samples were in the acidic range of the pH value with fermented milk sample being the most acidic. Similar pH values were reported by Lawal and Adedeji (2013). However, fresh cow milk has a pH value ranges from 6.6±0.11 to 6.8±0.09 (O'Connor, 1995; FAO, 1999). The results also showed that there was significant difference in the pH values between the locations of sample collection as well as the fermentation status. This may be as results of differences in both milking and processing procedures between the three locations of sampling.

The result of the analysis of temperature of the milk samples showed that there was significant difference in the temperature of the samples ($P < 0.05$) between the locations of collecting samples and also between fresh and fermented milk samples used in this study. The mean temperature 21.35±1.9^{0}C of the fresh milk samples collected for this study is less than 22.83±1.22^{0}C reported by Teshome *et al.,* (2015). Fresh milk samples obtained from Tashan Shongo market was significantly higher in temperature than those obtained from Gombe main market and Tashan Dukku market. This might be due to variations in the milk handling

equipments and also handling techniques. In the study area lack of cooling system and inefficient use of refrigerator by milk sellers increased the temperature of the milk samples in this study. This could contribute to the increased number of microbial contaminants (Teshome *et al.,* 2015).

Studies on the turbidity of both fresh and fermented milk samples indicated that there was significance difference ($P < 0.05$) between in the turbidity between fresh and fermented milk samples and also between the locations of collecting samples. Samples collected from Tashan Shongo nono market have the highest turbidity with 14.32±3.89NTU while Tashan Dukku market has the lowest turbidity with 3.80±2.60NTU. However, both fresh and fermented milk samples fail to satisfy the turbidity standard value of zero (0) which said that milk is considered sterile when it shows no turbidity (FAO, 2010).

The acidity content of both the fresh and fermented milk samples was found to be significantly different. The result of the analysis showed that there was significant difference in the total titratable acidity between the locations of sample collection. The titratable acidity in fermented milk 0.16±0.04 is significantly higher than that of fresh milk samples 0.15±0.03. The total Titratable acidity greater than 0.16±0.04 indicated that the milk samples were kept at room temperature for a longer period of time and poor handling (Teshome *et. al.,* 2015). Higher acidity value (0.194 ±0.006) was reported by Teshome *et al.*, (2015). Asaminew and Eyassu (2011) reported a higher acidity for milk samples collected individual farmers (0.023 ± 0.01). Similarly Zelalem and Faye (2006) also reported higher acidity (0.27).

There was no significant difference between fresh and fermented milk in the total aerobic mesophilic bacterial count but the results showed a significant difference between the locations of sampling. The aerobic plate count of fermented milk range from $6.53x10^8$CFU/mL to $9.18x10^8$CFU/mL, Gombe main market has the highest value of $9.18x10^8$CFU/mL. The results

also indicated that aerobic plate count of fresh milk range from $5.18x10^8$CFU/mL to $8.73x10^8$CFU/mL, Gombe main market having the highest number of aerobic plate count of $8.73x10^8$CFU/mL. Therefore, from both fresh and fermented milk samples, Gombe main market has the highest number while Tashan Dukku market has the lowest aerobic mesophilic plate count. The average mean is $4.22x10^9$CFU/mL; this value significantly higher than $3.2x10^4$CFU/mL reported by (Godic Torkar and Golc Teger, 2008) and $5.5x10^6$ CFU/mL reported by Aaku *et al*., (2004). This high number in Aerobic mesophilic plate count may be due to either low microbiological quality of the milk under process and/or contamination after heating process in fermented milk (Godic Torkar and Golc Teger, 2008). However, if these types of milk are consumed without taking proper attention to pasteurization then there will be a serious health problem.

Analysis on the anaerobic bacteria count revealed that there was no significance difference in the anaerobic count between fresh and fermented milk samples as well as between the locations of collecting samples. The findings revealed that fresh milk samples have the highest number of anaerobic bacteria than the fermented milk samples with $7.62x10^3$cfu/mL and $6.15x10^3$cfu/mL respectively. However, all the samples analyzed falls within the recommended/ legal limit of pasteurized and non-pasteurized milk which is $2.0x10^4$cfu/mL and $1.0x10^5$cfu/mL respectively FDA (2007).

The results demonstrated that fresh milk sample has the higher number of *Mycobacterium bovis* isolate than the fermented milk. The results also showed that the incidence of *Mycobacterium bovis* in both fresh and fermented milk is significantly different. The incidence of *Mycobacterium bovis* ranges from 21(23.33%) in fresh milk samples with Gombe main market has the highest number of *Mycobacterium bovis* isolate to 6(6.67%) in fermented milk samples.

This result is higher than the result reported by Al-Saqur *et al.*, (2009) 7(10.2%), Cadmus *et al.,* (2010) 4(1.0%), and Ben Kahla *et al.*, (2011) 5(4.9 %). The results of the study revealed that proper pasteurization of milk can remove and destroy *Mycobacterium bovis* since fermented milk have little or no micro organism and proper pasteurization of milk can assured the consumers the safety of milk and milk products they consumed.

Analysis of the milk samples for assessing the total number of coliform bacteria in the samples by means of coliform count showed that there was a significant difference between the locations of collecting samples as well as between fresh and fermented milk samples analyzed. The average mean of 7.22±4.6MPN/100mL obtained from fresh milk samples is greater than 6.57±6.4MPN/100mL reported by Okeke *et al.,* (2014); while the mean average 6.10±2.2MPN/100mL obtained from fermented milk is lower than 6.22±6.1MPN/100mL reported by Okeke *et al.,* (2014). The high coliform count obtained in this study could be a result of poor hygienic method of milking practice by local producers.

Studies on the moisture content of fresh and fermented milk samples obtained from various locations was conducted. Samples collected from Gombe main market have the highest; moisture content with 26.32±26.8mg/100mL while those collected from Tashan Dukku market has the lowest moisture concentration with 4.88±4.4mg/100mL. There was significance difference in the moisture content between the location of collecting samples and also between fresh and fermented milk samples. Moisture content is a measure of the water content in a product sample (Lawal and Adedeji 2013). The fermented milk samples have the highest moisture contents with 25.18±20.9mg/100mL while fresh has the lowest with 1.07±0.2mg/100mL moisture content. According to Aworh and Akinniyi, (1999), the moisture content accounts for the textural property of the product sample. The significant of moisture content in milk is that, high moisture

content implies high water activity which supports microbial growth consequently reducing the shelf life of the milk sample. Low moisture contents on the other hand, implies low water activities, low water activities causes reduction in microbial growth and the predominant microbial culture consequently increasing the shelf life of the milk samples as a result of low availability of water for microbial growth (Ajai, *et al.,* 2012).

The protein content of milk is an essential feature of its market value since higher protein content enhances performance of technological transformation Ponka *et al.*, **(**2013).There was significant difference in protein content between the locations of collecting sample and also between fresh and fermented milk samples. The results indicated that fresh milk samples have high percentage of protein with 51.88±11.2mg/100mL than fermented milk samples with 8.92±14.5mg/100mL. This is due to the fact that most of the proteins are destroyed by heat during pasteurization of the milk. The results agree with the work done of Lawal and Adedeji (2013). The values obtained in this study were higher than that found by Ahmad *et al*., (2008) in raw cow milk; this may be as a result of differences in the types of food used to feed the animals.

The results of the analysis showed that there was significant difference in the fat content between the locations of sample collection and also between fresh and fermented milk samples. Fresh milk samples have high percentage fat contents with 9.47±1.4mg/100mL than the fermented milk samples with 3.34±2.3mg/100mL. This is because the pastoralist used to remove fat during fermentation of the milk as such the amount of fat present the fermented milk is less than those present in the fresh milk. According to European Union quality standards for unprocessed whole milk, fat contents should not be less than 3.5mg/100ml (Tamime, 2009). Consequently, percentage of fat obtained in fresh milk samples fall within the recommended standard while those of fermented milk samples is very close to the recommended standard. The percentage fat content obtained is higher than those reported by Teshome *et al.*, (2015).

The study shows that there was no significant difference in ash contents between the locations of collecting sample as well as the fermentation status. Samples collected from Gombe main market have the highest ash content with 2.73±1.9mg/100mL and those collected from Tashan Shongo market has the lowest ash contents with 2.29±1.8mg/100mL. The mean ash content for both fresh and fermented milk samples was 2.48±1.9mg/100mL. This value is lower than 7.2mg/100mL reported by Lawal and Adedeji (2013). The value is higher than the value reported by Ponka *et al.*, **(**2013); Sanz Ceballos *et al.,* (2009). The amount of ash present in the milk sample determine the mineral elements that could be found in the milk samples, low concentration of ash indicated that there is low concentration of minerals elements in the milk samples.

The carbohydrate content showed that samples obtained from Tashan Dukku market have the highest carbohydrate contents with 56.49±22.8mg/100mL followed by those obtained from Tashan Shongo market with 48.61±21.5mg/100mL and then those from Gombe main market with 37.68±12.7mg/100mL. The result also showed that there was significant difference in the carbohydrate content between the location of collecting samples and also between fresh and fermented milk samples. The Average Carbohydrate content for both fresh and fermented milk samples is 47.59±20.9mg/100mL which is higher than (4.27mg/100mL) the value reported by Lawal and Adedeji (2013) and the carbohydrate contents reported by Ajai, *et al.,* (2012) ranged from 9.10 to 22.27mg/100mL which is lower than the value obtained from this study. Significantly, carbohydrate is essential to the body by providing energy needed by the body for its metabolic activities.

Magnesium concentration between the locations of sampling and also between fresh and fermented milk did not differ significantly. The result of the analysis showed that fermented milk

has high concentration of magnesium with 18.72±20.6mg/100mL than fresh milk samples with 15.41±2.0mg/100mL. The value obtained was lower than the value reported by Ponka *et al.*, **(**2013). This result is higher than 5.12mg/100mL reported by Lawal and Adedeji (2013).

Samples obtained from Tashan Shongo market have the highest manganese content with 11.60mg/100mL followed by those obtained from Gombe main market with 8.22mg/100mL and then those obtain from Tashan Dukku market with 8.87mg/100mL. The average of the manganese concentration of all the samples was 9.56mg/100mL, the value lower than 12.7-13.7mg/100mL reported by Ajai, *et al.,* (2012).

No significant difference in calcium content between the sampling locations was observed. However, it is significantly different ($P < 0.05$) between fresh and fermented milk samples. The calcium content is highest in samples collected from Tashan Shongo market with an average mean of 266.8mg/100mL. The lowest is Gombe main market with 246.07mg/100mL. There is also an overall mean value of 251.40mg/100mL of calcium content. This value is lower than the value reported by Ajai, *et al.,* (2012) which ranged between 2000.10mg/100mL to 2830.50mg/100mL. The values are in line with the values reported by Dirienzo, (2001) in Whey and milk products which ranged between 500mg/100mL to 2000mg/100mL.

Both the samples obtained from Tashan Dukku market and those obtained from Tashan Shongo market have 160.00mg/100mL which is highest value of potassium (K) content from the samples, while samples collected from Gombe main market had 159.57mg/100mL which is the lowest value obtained. The overall average mean of the potassium (K) content is 159.86mg/100mL. This value was lower than the value reported by Ajai, *et al.,* (2012) in Minna Nigeria which ranged between 1065.50mg/100mL to 1611.44 mg/100mL. The low value obtained may results from the type of feed used by the animals.

The samples obtained from Tashan Shongo market have the highest concentration of sodium with 250.42mg/100mL followed by Tashan Dukku market with 249.92mg/mL while samples collected from Gombe main market have the lowest value 230.73mg/100mL sodium concentration. The overall average mean of sodium content is 243.69mg/100mL. This value is lower than the value 293±49.39mg/100mL reported by Ponka *et al.*, **(**2013). This value is higher than 2.23mg/100mL reported by Lawal and Adedeji (2013)

Vitamin A concentration of the samples between the locations of collecting samples was remarkably different and also between fresh and fermented milk samples. The results of the analysis also showed that fresh milk samples have high concentration of vitamin A with 169.45±24.3IU/100mL than fermented milk samples with 60.43±25.4IU/100mL. The concentration of vitamin A obtained is 114.94±60.0 IU/100mL. This value is significantly lower than 264.5IU/100mL reported by Yasmin *et al.*, (2012). Vitamin A is important in normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions (Achikanu *et al*., 2013).

The result showed that there was significant difference in the vitamin C content between the locations of collecting samples and also between fresh and fermented milk samples. The results of the analysis indicated that fresh milk samples have high vitamin C concentration with 1.71±0.4mg/100mL than the fermented milk samples with 0.69±0.2mg/100mL. The concentration of vitamin C is obtained in this study is lower than those reported by Yasmin *et al.*, (2012). Vitamin C is a potent antioxidant that facilitates the transport and uptake of non-heme iron at the mucosa, the reduction of folic acid intermediates and the synthesis of cortisol. It is deficiency of vitamin C fragility to blood capillaries, gum decay and scurvy (Achikanu *et al*., 2013).

The vitamin E contents of both fresh and fermented milk samples range from 0.33±0.20mg/100mL to 0.66±0.27mg/100mL in fermented and fresh milk respectively. The result showed that fermented milk is higher with 0.66±0.27mg/100mL than fresh milk samples with 0.33±0.20mg/100mL. The result obtained in this study is higher than the result reported by Yasmin *et al*., (2012). The result also showed that there was significant difference in the vitamin E content obtained between the locations of collecting samples and also between fresh and fermented milk samples. Vitamin E is a powerful antioxidant which helps to protect cells from damage by free radicals and it is vital to the formation and normal function of red blood cell and muscles (Lukaski, 2004).

5.1 Conclusion

The predisposing factors to contamination are promoted by the environment, health of the animals and the cleanliness of the pastoralist. The milk contained physico-chemical parameters that are in-line with the internationally acceptable limit. The number of aerobic mesophilic bacteria found in this study is greater than $1.0x10^5$cfu/mL and $2.0x10^4$cfu/mL which is recommended by FDA (2007). However, in terms of microbial count of fresh and fermented milk, there is no choice between fresh and fermented milk because there is no significant difference between fresh and fermented milk samples analyzed. However, the fact that there was contamination with *M. bovis* at 7 - 23% of both fermented and fresh milk respectively, milk vendors in Gombe leaves much to be desired in terms of microbiological quality of the product. Coliform bacterial count revealed that the milk samples contained values within the acceptable limit of 10MPN/100mL and hence the milk is said to be safe in terms of contamination by coliform bacteria In spite of the fact that both fresh and fermented milk examined contained an appreciable content of calcium, potassium, sodium, and magnesium but least in manganese

although within the recommended values. They also contained vitamins in good quantity of vitamin A, vitamin E and vitamin C.

5.2 Recommendations

1. There is need for people to abide by the rules and regulations in milking and they should ensure a good hygiene of their environment and their animals.

2. There is a need for pasteurization of milk before consumption to prevent zoonotic transmission of tuberculosis.

3. Accurate measured should be taken during milking and also during processing of the milk to form fermented milk.

4. There is also a need to create awareness among Fulani's on the importance of good hygiene practice and also the dangers associated with *Mycobacterium bovis*.

5. Clinicians and veterinarians should be aware of the occurrence of *M. bovis* in the stable diet of residents (Milk) in the study area for proper diagnosis and treatment.

6. Government should educate Fulani's on how they will prevent themselves and their cattle from bovine tuberculosis.

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APPENDICES

APPENDIX I: Analysis of the Significant Variation in the pH of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Corrected Model	236.727[a]	5	47.345	1.027E3	.000
Intercept	5556.444	1	5556.444	1.205E5	.000
Location	1.223	2	.612	13.264	.000
Fermentation	234.202	1	234.202	5.080E3	.000
Location * Fermentation	1.303	2	.651	14.129	.000
Error	8.022	174	.046		
Total	5801.194	180			
Corrected Total	244.750	179			

a. R Squared = .967 (Adjusted R Squared = .966)

APPENDIX II Analysis of the Significant Variation in the Temperature of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5611.746[a]	5	1122.349	390.913	.000
Intercept	44957.323	1	44957.323	1.566E4	.000
Location	60.299	2	30.149	10.501	.000

Fermentation	5527.591	1	5527.591	1.925E3	.000
Location * Fermentation	23.857	2	11.928	4.155	.017
Error	499.571	174	2.871		
Total	51068.640	180			
Corrected Total	6111.317	179			

a. R Squared = .918 (Adjusted R Squared = .916)

APPENDIX III: Analysis of the Significant Variation in the Turbidity of the Milk Samples by ANOVA

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4356.650[a]	5	871.330	133.004	.000
Intercept	12852.450	1	12852.450	1.962E3	.000
Fermentation	893.339	1	893.339	136.364	.000
Location	3451.233	2	1725.617	263.407	.000

Fermentation * Location	12.078	2	6.039	.922	.400
Error	1139.900	174	6.551		
Total	18349.000	180			
Corrected Total	5496.550	179			

a. R Squared = .793 (Adjusted R Squared = .787)

APPENDIX IV: Analysis of the Significant Variation in the Total Titratable Acidity of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.064[a]	5	.013	14.874	.000
Intercept	4.480	1	4.480	5.211E3	.000

Location	.009	2	.004	5.069	.007
Fermentation	.007	1	.007	7.677	.006
Location * Fermentation	.049	2	.024	28.279	.000
Error	.150	174	.001		
Total	4.694	180			
Corrected Total	.214	179			

a. R Squared = .299 (Adjusted R Squared = .279)

APPENDIX V: Analysis of the Significant Variation in the Aerobic Bacterial Count of the Milk Samples by ANOVA

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	191.015[a]	5	38.203	2.647	.025
Intercept	5348.957	1	5348.957	370.618	.000
Location	163.904	2	81.952	5.678	.004
Fermentation	13.312	1	13.312	.922	.338
Location * Fermentation	13.800	2	6.900	.478	.621

Error	2511.262	174	14.433
Total	8051.234	180	
Corrected Total	2702.277	179	

a. R Squared = .071 (Adjusted R Squared = .044)

APPENDIX VI: Analysis of the Significant Variation in the Anaerobic Bacterial Count of the Milk Samples by ANOVA

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	21.528[a]	5	4.306	1.456	.207
Intercept	1507.427	1	1507.427	509.805	.000
Fermentation	1.861	1	1.861	.629	.429
Location	6.536	2	3.268	1.105	.333
Fermentation * Location	13.131	2	6.566	2.220	.112
Error	514.496	174	2.957		

Total	2043.450	180
Corrected Total	536.023	179

a. R Squared = .040 (Adjusted R Squared = .013)

APPENDIX VII: Analysis of the Significant Variation in the Total Coliform Count of the Milk Samples by ANOVA

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	466.628[a]	5	93.326	8.676	.000
Intercept	7986.672	1	7986.672	742.470	.000
Location	187.911	2	93.956	8.734	.000
Fermentation	56.672	1	56.672	5.268	.023
Location * Fermentation	222.044	2	111.022	10.321	.000
Error	1871.700	174	10.757		
Total	10325.000	180			

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	466.628[a]	5	93.326	8.676	.000
Intercept	7986.672	1	7986.672	742.470	.000
Location	187.911	2	93.956	8.734	.000
Fermentation	56.672	1	56.672	5.268	.023
Location * Fermentation	222.044	2	111.022	10.321	.000
Error	1871.700	174	10.757		
Total	10325.000	180			
Corrected Total	2338.328	179			

a. R Squared = .200 (Adjusted R Squared = .177)

APPENDIX VIII: Analysis of the Significant Variation in the Moisture Content of the Milk Samples by ANOVA

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	58140.743[a]	5	11628.149	297.190	.000
Intercept	31020.414	1	31020.414	792.813	.000
Location	15993.948	2	7996.974	204.385	.000
Fermentation	26165.860	1	26165.860	668.741	.000
Location * Fermentation	15980.935	2	7990.467	204.219	.000
Error	6808.103	174	39.127		
Total	95969.260	180			
Corrected Total	64948.846	179			

a. R Squared = .895 (Adjusted R Squared = .892)

APPENDIX IX: Analysis of the Significant Variation in the Protein Content of the Milk Samples by ANOVA

Source	Type III Sum of Squares	Difference	Mean Square	F	Sig.
Corrected Model	87861.741	5	17572.348	131.982	.000
Intercept	163609.578	1	163609.578	1.229E3	.000
Location	3841.748	2	1920.874	14.427	.000
Fermentation	81149.553	1	81149.553	609.496	.000
Location * Fermentation	2870.440	2	1435.220	10.780	.000
Error	23166.719	174	133.142		
Total	274638.038	180			
Corrected Total	111028.459	179			

a. R Squared = .791 (Adjusted R Squared = .785)

APPENDIX X: Analysis of the Significant Variation in the Fat Content of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2097.828[a]	5	419.566	309.239	.000
Intercept	7393.294	1	7393.294	5.449E3	.000
Location	234.262	2	117.131	86.331	.000
Fermentation	1690.348	1	1690.348	1.246E3	.000
Location * Fermentation	173.219	2	86.610	63.835	.000
Error	236.077	174	1.357		
Total	9727.200	180			
Corrected Total	2333.906	179			

a. R Squared = .899 (Adjusted R Squared = .896)

APPENDIX XI: Analysis of the Significant Variation in the Ash Content of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Corrected Model	9.498[a]	5	1.900	.522	.759
Intercept	1106.229	1	1106.229	304.131	.000
Location	6.056	2	3.028	.833	.437
Fermentation	.051	1	.051	.014	.906
Location * Fermentation	3.391	2	1.695	.466	.628
Error	632.898	174	3.637		
Total	1748.625	180			
Corrected Total	642.396	179			

a. R Squared = .015 (Adjusted R Squared = -.014)

APPENDIX XII: Analysis of the Significant Variation in the Carbohydrate Content of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	47861.097[a]	5	9572.219	54.636	.000

Intercept	407680.687	1	407680.687	2.327E3	.000
Location	10709.796	2	5354.898	30.564	.000
Fermentation	27975.200	1	27975.200	159.676	.000
Location * Fermentation	9176.101	2	4588.050	26.187	.000
Error	30484.842	174	175.200		
Total	486026.626	180			
Corrected Total	78345.939	179			

a. R Squared = .611 (Adjusted R Squared = .600)

APPENDIX XIII: Analysis of the Significant Variation in the Magnesium Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1644.467[a]	5	328.893	1.550	.177
Intercept	52428.800	1	52428.800	247.019	.000

Location	504.933	2	252.467	1.190	.307
Fermentation	493.356	1	493.356	2.324	.129
Location * Fermentation	646.178	2	323.089	1.522	.221
Error	36930.733	174	212.246		
Total	91004.000	180			
Corrected Total	38575.200	179			

a. R Squared = .043 (Adjusted R Squared = .015)

APPENDIX XIV: Analysis of the Significant Variation in the Manganese Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16990.136[a]	5	3398.027	16.163	.000
Intercept	16464.235	1	16464.235	78.313	.000
Location	386.534	2	193.267	.919	.401

Fermentation	16216.513	1	16216.513	77.135	.000
Location * Fermentation	387.089	2	193.545	.921	.400
Error	36581.155	174	210.237		
Total	70035.525	180			
Corrected Total	53571.290	179			

a. R Squared = .317 (Adjusted R Squared = .298)

APPENDIX XV: Analysis of the Significant Variation in the Calcium Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	87745.248[a]	5	17549.050	4.680	.000
Intercept	1.134E7	1	1.134E7	3.025E3	.000
Location	8376.708	2	4188.354	1.117	.330

Fermentation	68094.450	1	68094.450	18.161	.000
Location * Fermentation	11274.090	2	5637.045	1.503	.225
Error	652395.799	174	3749.401		
Total	1.208E7	180			
Corrected Total	740141.048	179			

a. R Squared = .119 (Adjusted R Squared = .093)

APPENDIX XVI: Analysis of the Significant Variation in the Potassium Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	95902.811[a]	5	19180.562	79.053	.000
Intercept	4599683.756	1	4599683.756	1.896E4	.000
Location	7.511	2	3.756	.015	.985
Fermentation	95772.800	1	95772.800	394.730	.000

Location * Fermentation	122.500	2	61.250	.252	.777
Error	42217.433	174	242.629		
Total	4737804.000	180			
Corrected Total	138120.244	179			

a. R Squared = .694 (Adjusted R Squared = .686)

APPENDIX XVII: Analysis of the Significant Variation in the Sodium Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	905017.111[a]	5	181003.422	5.057	.000
Intercept	1.069E7	1	1.069E7	298.638	.000
Location	15113.678	2	7556.839	.211	.810
Fermentation	885363.200	1	885363.200	24.736	.000

Location * Fermentation	4540.233	2	2270.117	.063	.939
Error	6227999.467	174	35793.100		
Total	1.782E7	180			
Corrected Total	7133016.578	179			

a. R Squared = .127 (Adjusted R Squared = .102)

APPENDIX XVIII: Analysis of the Significant Variation in the Vitamin C Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	48.098[a]	5	9.620	138.435	.000
Intercept	259.440	1	259.440	3.734E3	.000
Location	1.153	2	.576	8.295	.000
Fermentation	46.614	1	46.614	670.827	.000

Location * Fermentation	.331	2	.165	2.380	.096
Error	12.091	174	.069		
Total	319.629	180			
Corrected Total	60.189	179			

a. R Squared = .799 (Adjusted R Squared = .793)

APPENDIX XIX: Analysis of the Significant Variation in the Vitamin A Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	555206.612[a]	5	111041.323	215.411	.000
Intercept	2378085.613	1	2378085.613	4.613E3	.000
Location	14400.400	2	7200.200	13.968	.000
Fermentation	534808.513	1	534808.513	1.037E3	.000

Location * Fermentation	5997.700	2	2998.850	5.818	.004
Error	89694.525	174	515.486		
Total	3022986.750	180			
Corrected Total	644901.138	179			

a. R Squared = .861 (Adjusted R Squared = .857)

APPENDIX XX: Analysis of the Significant Variation in the Vitamin E Concentration of the Milk Samples by ANOVA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.375[a]	5	1.075	19.462	.000
Intercept	43.857	1	43.857	794.042	.000
Location	.366	2	.183	3.315	.039
Fermentation	4.740	1	4.740	85.821	.000

Location * Fermentation	.268	2	.134	2.428	.091
Error	9.611	174	.055		
Total	58.843	180			
Corrected Total	14.985	179			

a. R Squared = .359 (Adjusted R Squared = .340)

APPENDIX XXI: Standard Limits of Coliform Count and Total Bacterial Count in Milk and Quality Judgments

Coliform count MPN/mL	Milk quality	Total bacterial count	Milk quality
<10	Achievable (Desirable)	$<10^5$	Acceptable (good)
10 – 50	Very good	10^5 - 5 x10^5	Moderate
50 – 100	Acceptable(good)	>5 x10^5	Poor
100 – 500	Substandard		
>500	Poor (Rejected)		

Source: APHA, 1992

APPENDIX XXII: Standard Values for Proximate Composition of Milk

Proximate	Recommended value (mg/100mL)
Moisture	87.3
Protein	2.9
Fat	4.0
Ash	0.7
Carbohydrate	4.7

Source: Food Standards Agency (2002)

APPENDIX XXIII: Standard Values for Vitamin Concentrations of Milk

Vitamins	Recommended value
Vitamin A	46 IU/100mL
Vitamin C	2 mg/100mL
Vitamin E	0.08 mg/100mL

Source: Food Standards Agency (2002)

APPENDIX XXIV: Standard Values for the Concentrations of Mineral Elements of Milk in mg/100mL

Proximate	Recommended value (mg/100mL)
Magnesium	11
Manganese	Trace
Calcium	112
Potassium	160
Sodium	4.4

Source: Food Standards Agency (2002)

APPENDIX XXV: MPN Index for Various Combinations of Positive Results When Five Tubes are used per Dilution

Number of positive tubes			MPN index per 100 mL	Number of positive tubes			MPN index per 100 mL
First dilutions	Second dilutions	Third dilutions		First dilutions	Second dilutions	Third dilutions	
0	0	1	< 2	4	2	1	26
0	1	0	2	4	3	0	27
0	2	0	4	4	3	1	33
1	0	0	2	4	4	0	34
1	0	1	4	5	5	0	23
1	1	0	4	5	0	1	30
1	1	1	6	5	0	2	40
1	2	0	6	5	1	0	30
2	0	0	4	5	1	1	50
2	0	1	7	5	1	2	60
2	1	0	7	5	2	0	50
2	1	1	9	5	2	1	70
2	2	0	9	5	2	2	90
2	3	0	12	5	3	0	80
3	0	0	8	5	3	1	110

3	0	1	11	5	3	2	140
3	1	0	11	5	3	3	170
3	1	1	14	5	4	0	130
3	2	0	14	5	4	1	170
3	2	1	17	5	4	2	220
4	0	0	13	5	4	3	280
4	0	1	17	5	4	4	350
4	1	0	17	5	5	0	240
4	1	1	21	5	5	1	300
4	1	2	26	5	5	2	500
4	2	0	22	5	5	3	900
				5	5	4	1600
				5	5	5	>1600

Source: APHA, 1992