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Udder health inflammatory markers in camel milk (*Camelus dromedarius*) and milk yield
Juverhälsoinflammatoriska markörer i kamelmjölk (Camelus dromedarius) och mjölkmängd

Photo: Sofie Tinggren

Sofie Tinggren

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Supervisor: Jane Morrell SLU, Department of Clinical Sciences

Co Supervisors: Ann Nyman, Dinah Seligsohn, Kerstin De Verdier SLU, Department of Clinical Sciences

External mentor: Mario Younan, National Veterinary Institute (SVA)

Examiner: Karin Östensson SLU, Department of Clinical Sciences

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Abstract

Kenya is one of the biggest producers of camel milk in the world. Apart from milk production, camels are also a very important source of food and income for pastoralists. Camels (*Camelus dromedarius*) are well adapted to the harsh environments and arid parts of the country. Mastitis is one of the most common and costly diseases of dairy animals because of loss in milk yield and cost of treatment. The quality of the milk also decreases due to mastitis and the milk will be worth less. Mastitis can affect the storage life of the milk, which can lead to a loss in income. The aim of this literature review was to obtain a greater understanding of why camel milk has become so popular and what challenges the milk industry in Kenya must overcome. The aim of the field study was to investigate if there were any associations between the inflammatory markers, somatic cell count (SCC), N-acetyl-B-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH), udder skin temperature or the California mastitis test (CMT), and subclinical mastitis or decreased quarter milk yields in affected quarters in camels. Descriptive statistic of the distribution of the inflammatory markers and milk yield were performed as well as statistical analyses of associations between each inflammatory marker and milk yield. The inflammatory markers SCC, NAGase, LDH and CMT appeared to be good markers for subclinical mastitis in *Camelus dromedarius*. The udder skin temperature did not work well as a marker for subclinical mastitis in this study. Milk yield did not show any relationship with CMT or with SCC. The percentage difference in milk yield between paired udder quarters nevertheless indicated that a high CMT was associated with a decreased milk yield up to 44.7%. However, more research is needed. As CMT is an easy and cheap way of detecting udder quarters with subclinical mastitis, it could be used as a measurement to improve udder health and camel milk quality in pastoral camel herds in Kenya.

Sammanfattning

Kenya är en av de största producenterna av kamelmjölk i världen. Förutom mjölkproduktion är kameler också en mycket viktig källa till mat och inkomst för pastoralister. Kameler (*Camelus dromedarius)* är väl anpassade till de hårda miljöerna och de torra delarna av landet. Mastit är en av de vanligaste och mest kostsamma sjukdomarna inom mjölkproduktionen pga den förlorade mjölkmängden och kostnaden för behandlingar. Mjölkens kvalitet minskar också pga mastit och mjölken blir mindre värd. Mastit kan också påverka mjölkens lagringstid, vilket kan leda till inkomstförluster. Syftet med denna litteraturöversikt var att få större förståelse för varför kamelmjölk har blivit så populär och vilka utmaningar mjölkindustrin i Kenya måste övervinna. Syftet med fältstudien var att undersöka om det fanns några samband mellan de inflammatoriska markörerna, somatisk cell count (SCC), N-acetyl-B-D-glukosaminidase (NAGase) och laktatdehydrogenase (LDH), juverhudstemperaturen eller California mastit test (CMT), och om de hade en relation med subklinisk mastit eller minskad mjölkmängd på juverdels nivå hos kameler. Beskrivande statistik över fördelningen av de inflammatoriska markörerna och mjölkmängd utfördes såväl som statistiska analyser eller med föreningar mellan varje inflammatorisk markör och mjölkmängd. De inflammatoriska markörerna SCC, NAGas, LDH och CMT är bra markörer för subklinisk mastit för Camelus dromedarius. Hudtemperaturen fungerade inte som en markör för subklinisk mastit i denna studie. Mjölkmängden visade inte något samband med CMT eller SCC. Den procentuella skillnaden i mjölkmängd mellan parvis jämförda juverdelar visade dock att ett högt CMT var förknippat med en reducerad mjölkmängd på upp till 44,7%, men mer forskning behövs. Eftersom CMT är ett enkelt och billigt sätt att hitta juverdelar med subklinisk mastit, kan det användas av pastoralister i kamelbesättningar, som en mätning för att förbättra hälsan och kvaliten på kamelmjöl i Kenya.

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Introduction

Dromedary camels (*Camelus dromedarius*) are well adapted to hot climates and arid environments due to their unique physiological, anatomical and behavioural characteristics. Camelids originate from North America. There are two groups of camelids: one which migrated to the west through the land connection between America and Asia, today known as the Bering straits, and the other group which migrated south and eventually developed into the South American camelids, SACs, or New World Camels (NWC). They all have the ability to ruminate (Odöö and Bornstein, 1993) but unlike the true ruminants such as cows, sheep or goats that have four "stomachs", the camelids have only three "stomachs" (Ross *et al.,* 1979). The western camels developed into the one-humped camels (*Camelus dromedarius*) and the two-humped camels (*Camelus bactrianus*) and (*Camelus ferus*), whereas the camels that migrated south, which do not have a hump, developed into the llamas, alpaca, vicuna and guanaco,. Llamas and alpacas are domesticated animals, used as pack animals and for meat and wool production, while vicuna and guanaco are mostly found in the wild (Odöö and Bornstein, 1993). The Dromedary camels are able to cope with hot, dry weather, and are mostly found in the northern and eastern parts of Africa, the Middle East and Central Asia. The Bactrian camels are better able to cope with cold weather and are mainly concentrated in China and Mongolia. The Old World Camels (OWC) provide several products as milk, meat, wool, bone and dung, as well as being working animals on farms, for carrying goods, riding and also for tourism (Odöö and Bornstein, 1993).

The camel population is estimated to be about 25 million; of these 95% are dromedaries (Faye, 2013). They provide milk which is a very important product for the nomadic pastoralist economy (Musinga *et al.,* 2008). The dromedary camel breed Rendille can produce more milk than four Zebule cows during the dry season and camel milk has a higher economic value than cow milk (Spencer, 1973). Camels are mainly browsers and with their height they are able to reach feed that other livestock cannot; thus they do not compete for forage with other ruminant livestock (Odöö and Bornstein, 1993). With a decreasing food production in Africa per capita and an increasing human population, keeping dairy camels is a sustainable way to develop food production in semi-arid and arid lands (Schwartz *et al*., 1992)

This study is a student project for a master thesis. The thesis was developed from an on-going PhD project with the title: "Control of *Streptococcus agalactiae* to reduce subclinical mastitis in pastoralist camel herds in Kenya". The sampling and practical work for this master thesis were performed in Laikipia district in Kenya. The aim of the study was to investigate if there were any associations between the inflammatory markers, somatic cell count (SCC), N-acetyl-B-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH), udder skin temperature or the California mastitis test (CMT), with subclinical mastitis or decreased quarter milk yields in affected quarters in camels. The methods that were used were both existing, well-tried methods, and new but not yet so well-recognized methods described in studies of udder inflammation in camels. The aim of the literature study was to review these inflammatory markers and, in addition, explain why camel milk has become so popular and what challenges the milk industry in Kenya has to overcome.

Literature review

Background

Kenya is one of the biggest producers of camel milk in the world (FAO 2018). Camels are well adapted to harsh environments and to the arid parts of the country. They have also become a very important food and income source for the pastoralists with the increasing commercialization of camel milk (FAO 2018). The camel industry in the Isiolo district in Kenya has grown considerably in the last three to four decades as pastoralists are increasingly using their camels for commercial milk production. This development has arisen because camels are very mobile animals, which is necessary in areas with dry climate and poor feed (Musinga *et al.,* 2008).

More than 70% of Kenya consist of arid and semi-arid land, which is poorly suited to agricultural farming (Ominde *et al.,* 1988). About 10% of Kenya´s population are pastoralist (Kirkbride & Grahn, 2008). In the past, pastoralists mainly herded cattle and small livestock, but due to changes in the climate with long and hard droughts, the one-humped camel (*Camelus dromedarius*) has become more common as a climate-resistant dairy animal (Faye, 2012). During the severe drought in Kenya in 1984, pastoralists who were concentrating on camel production lost fewer animals than the households that kept cattle, goats and sheep (Fratkin et al., 1990). Another severe drought in Kenya led to huge losses in the livestock industry. Death rates from 40 to 70% for sheep, goats and cattle were recorded after the drought (Serena, 2011).

The camel's ability to cope with a very dry climate is due to its capacity to save body water, which stems from both behavioural and anatomical adaptations (Wilson, 1998). Camels prefer to browse during the night when the temperature is lower. However, this practice is not common among herded camels for safety and practical reasons (Wilson, 1998). The camel's body fat is concentrated in the hump instead of covering the whole body, making it easier to reduce body heat loss from the skin surface. The camel can also change its body temperature up to 6°C depending on the outside temperature, resulting in reduced water losses from the surface of the skin (Wilson, 1998). In addition, the camel can recycle water, by absorbing it from both faeces and urine. The camel does lose its appetite and milk yield when dehydrated, but these occur after a week of dehydration (Bekele *et al.,* 2011), in contrast to cattle which lose their appetite and milk yield after one day of dehydration (Steiger Burgos *et al.,* 2001). Camels can produce much more milk than cattle during dry conditions (Spencer, 1973).

The increased interest in, and demand for, camel milk in the last few decades have prompted a need to transport the milk over long distances. However, longer transport requires better milk quality (Musinga *et al.,* 2008). A common condition of the udder that affects the quality of milk is mastitis. Mastitis is defined as an inflammation of the udder, most often caused by infection by microorganisms, for example, due to an unhygienic environment or due to physical injuries in the udder (Sandholm *et al.,* 1995). Mastitis is one of the costliest diseases in the dairy cow industry because of the loss in milk yield, the cost of treatment with antibiotics and the cost of culling due to chronic inflammation (Jingar, 2017). In a study by Sinha *et al.* (2014), financial losses due to unsold bovine milk were caused by decreased yield and the decreased quality of the milk. In a study by Ma *et al.* (2000) it was shown that mastitis infections also affect the storage life of the milk, which can lead to a loss of income. Somatic cell count (SCC) is used to measure the level of inflammation and is a common

health indicator for dairy animals (Sandholm *et al.,* 1995). When dairy cow milk with a SCC of 45,000 cells/mL was compared with milk that contained 849,000 cells/mL milk, a significant negative association was seen between level of inflammation and storage time, despite the fact that both sets of samples had been pasteurised and homogenised. Moreover the samples with the highest SCC had a rancid and bitter taste after 21 days´ storage (Ma *et al.,* 2000).

A study on the camel milk industry in Isiolo District by SNV Netherlands Development Organization (Musinga *et al.,* 2008) concluded that the camel milk industry can permanently change the way of life of the inhabitants of the arid and semi-arid parts of the country. The demand for camel milk is growing, both nationally and internationally. However, various players in the milk chain, as well as milk organizations, need to develop to be able to market more milk (Musinga *et al.,* 2008)

Camel milk industry in Kenya

The camel industry in Kenya has grown considerably in the last three to four decades as camels have become more frequently used for commercial milk production by pastoralists (Musinga *et al.,* 2008). Several factors make camel milk popular: for example, people who are sensitive to lactose or are lactose-intolerant can drink camel milk without any adverse side effects. The milk contains a low level of the protein β-casein compared to cow´s milk and lacks β-lactoglobulin. These proteins cause an allergic reaction in lactose-intolerant people (Konuspayeva *et al.,* 2009).

Camel milk is also a popular healing drink. Pastoralists have always drunk camel milk, both as daily food and medicine (Guliye *et al.,* 2007). There is now an increasing interest from other groups of people. The suggested curative effects of camel milk are presented to a greater public by marketing it in popular magazines and articles online. “Headlines” describing all the benefits are listed; for example, it is claimed that camel milk avoids allergies in children, helps to fight autoimmune disorders and autism, has anti-aging and anti-diabetic properties, can cure tuberculosis and is good for weight loss (Wells, 2018; Ameya, 2017; Dubey *et al.,* 2016; Hall, 2011)

The population in Kenya has increased from around 15 million people in the 1980s to 51.5 million people in 2018 (Worldometer 2018). The high growth of the population and a pronounced migration into urban areas from the countryside has led to a growing demand for both food and camel milk (Noor *et al.,* 2012). The neighboring country, Somalia, started a decade-long civil war in 1991, which caused many refugees to seek safety in Kenya (Hammond, 2018). Somalians have a long tradition of keeping camels and drinking their milk (Guliye *et al.,* 2007), and Somalia has the world´s largest dromedary population (Faye, 2014).

A questionnaire about “Reproduction and breeding in dromedary camels” was completed by camel-keeping pastoralists in southeastern Nigeria in a field study by Abdussamad *et al*. (2011). The gestation period was 12-13 months for camels and the average age for camels at first calving was between 4 and 5 years. Moreover, the pastoralist stated that the average lactation period for camels was 11.8 months, although lactation length could vary between 8 and 18 months. The calving interval was, on average, 23.8 months. Females can breed for more than 20 years and have 8-12 calves during that time (Abdussamad *et al.*, 2011). According to FAO (2018), camels in Africa produce, on average, 1000 to 2700 liters of milk

per lactation. The increasing use of camel milk and realization of its economic advantages in Africa were associated with an interest in reproduction and breeding (Atigui *et al.,* 2013).

In a study about the contribution of camels to the household diet of pastoralists, it was shown that half of the annual nutrition came from camels (Farah *et al.,* 2004). The household diet includes 84.5% of livestock products, with camel meat (24.6%) and milk (20.4%) making the biggest contribution, followed by 17.6% goat meat and 14.7% cow meat (Elhadi *at al.*, 2015). The average consumption of camel and cow milk varies depending on season. In the wet season, the consumption of camel and cow milk was 2.0 liters and 1.6 liters per day, respectively, while in the dry season the consumption of camel milk increases to 2.5 liters whereas cow milk decreased to 0.5 liters per day. Camel milk made the biggest contribution to the household diet in the dry season, at 28.2% of the intake. This is a consequence of a lack of supplies, for example, of vegetables, in the dry season (Elhadi *at al*., 2015).

The camel milk industry faces many challenges. Despite an expanding industry and a high demand, the hygienic aspects of food safety are not a primary concern for the majority of consumers (Musinga *et al.,* 2008). Raw camel milk is a traditional medical potion for many, and proper handling or boiling is not considered to be necessary. The potential to extend the camel milk industry, both for a broader range of customer and at a national level, requires a focus on hygiene (Musinga *et al.,* 2008).

Odongo *et al.* (2016) interviewed 235 people, including herdsmen and people from bulking and retailing centers, about camel milking routines in Isiolo town. The containers into which the milk is collected are made entirely of plastic: the reasons for this were stated to be because plastic containers are not as heavy as other materials (47%), or because plastic containers are inexpensive (36.3%). The lightness of the milking container is an important aspect for the milker as he holds it up with his knee due to the height of the camel´s udder. Also 17.3% of the dealers in the study by Odongo *et al.* (2016) claimed that plastic containers were good for preserving milk. After milking, the milk can be stored up to 11 hours before it reaches the first cooling station, often being carried by motorbike couriers in Isiolo town. The plastic milk containers are washed and held over the fire so that the number of microorganisms can be reduced by the smoke (Odongo *et al.,* 2016).

Odongo *et al.,* (2016) revealed many risk factors for microbial contamination along the milk chain. The study shows that the camel´s udder is often not wiped clean before milking and the hands of the milkers are normally not washed. The number of bacteria was lower on the camels´ teats than on the milkers´ hands. It was assumed that this was due to a cleansing action by the calf sucking to initiate milk let-down. However, it could also be that the calf introduces microbial contamination to the udder prior to milking (Noor *et al.,* 2013).

Another risk factor for bacterial contamination of camel milk is mixing of milk from healthy and mastitic udders. The overall opinion among participants in the report by Odongo *et al.* (2016) was that milk from sick camels is not a health risk and it would be a waste not to use it. The decision to use medication for a sick camel is often made by the herdsman himself without consulting a veterinarian. It is likely that milk from these camels contains antibiotic residues, which could lead to rejection at the milk collection center (Odongo *et al.,* 2016).

The camel industry in Kenya represents a market potential for pastoral women. The SNV

(Netherlands Development Organisation) conducted a case study about pastoral women in the Kenya camel milk chain and the challenges for pastoral women. The men milk the camels and are responsible for production, whereas the women are responsible for the milk and are active in the least profitable sections of the milk value chain, such as intermediary (80%), micro processing (30%) and local markets (30%) (Siloma, 2011).

One difficulty is the possibility for Kenyan women to obtain financial help from the bank. Many pastoral women are muslims and, according to the Islamic sharia rules, are not permitted to borrow money (Siloma, 2011). Another obstacle is payment for the milk. The transportation of milk for many women is done by public transportation, with the driver receiving the money for milk sold. The risks for the driver are high and robbery occasionally occurs (Siloma, 2011). Projects where money transfers are made to women by cell phone are being introduced to avoid robbery and to make the transactions more efficient. In addition, poor quality of camel milk is a challenge. Collaboration with herdsmen to improve milk hygiene is required (Siloma, 2011).

Another challenge in the camel milk industry is that the more commercialized production becomes, the closer to the dairy centers the camel herds need to be. Noor *et al.* (2012) used the expression "peri-urban production system" to describe how the pastoralist herding system is becoming more common closer to towns and cities. The closer the camel herds get to each other, the higher the density of the animals will be. Risks associated with a high density of camel herds close to each other include both that the vegetation will be over-exploited (Noor *et al.,* 2012) and that the transmission of disease will be higher between the herds (Bornstein, 2018).

Mastitis

Mastitis, inflammation of the udder, is often a result of a bacterial infection and can be classified as clinical, with visible symptoms, or subclinical, with non-visiblesymptoms. Clinical mastitis can be detected by visible signs, such as swellings of an udder quarter, changed milk consistency and colour, fever, etc. Subclinical mastitis is harder to detect as no visible changes can be seen. However, the milk composition is changed which can be confirmed by laboratory tests or cow/camel side tests (Guliye *et al.,* 2002). Clinical mastitis can be sub-classified depending on how severe the symptoms are (SVA 2018). In acute mastitis, the animal´s udder can be swollen and sore. The animal can be weak and slow, have a fever, and there are usually changes in the texture, color and odour of the milk. Milk yield can be reduced and the concentration of somatic cells in the milk will increase (Sandholm *et al.,* 1995). These symptoms are the same for several dairy animals, such as sheep (Gårdochdjur hälsa, 2018), goats (Svenska getavelsförbundet 2018a) and camels (Wilson, 1998). Untreated mastitis could develop into a chronic case. An acute clinical mastitis is often treated with antibiotics (SVA 2018). In severe cases of mastitis the cow may die or be culled (Jingar *et al.,* 2017). The prevalence of mastitis in milking camels in Kenya has been investigated in several studies (Toroitich *et al.,* 2017**,** Kaindi *et al.,* 2011; Abdurahman 1996). In Africa and in the Middle East the prevalence of clinical mastitis in camels varies from 24.1% (Almaw *et al.,* 2000) to 76.0% (Seifu *et al.,* 2010), and for subclinical mastitis in camels from 20.7% (Abera *et al.,* 2009) to 33% (Aljumaah *et al.,* 2011).

The camel has not previously been recognized as an animal for dairy research. As severe droughts are becoming more common and as demand on camel products increases, the need

for research is growing. However, most of the research on subclinical and clinical mastitis is focused on dairy cows. These dairy animals have physiological and anatomical similarities that make research on dairy cows applicable to dairy camels as well (Bornstein, 2018). The camel´s udder has four quarters, each having glands and gland cisterns as in the cow´s. The difference is that the camel has two glands per quarter each of which has its own separate "small" gland cisterns (Abshenas *et al.,* 2007), compared with the cow where there is only one gland and a "big" cistern per quarter (Sandholm *et al.,* 1995). Cow's milk has more lactose and protein than camel milk (Soliman, 2005). Camels have similar udder proportions to the cow (Bogucki 2017, Šlyžius *et al.,* 2013), with 60% of the milk yield originating from the hind udder quarters and 40% milk from the front udder quarters (Caja *et al.,* 2011, Eisa *et al.,* 2009). The biggest difference is the vitamin content, since camel milk has much more vitamin C than cow milk but lacks vitamin A. Camels are often compared with dairy cows when they are kept in the same environment (Faye, 2012).

The most common bacteria causing an elevated somatic cell count (SCC) in cows, sheep and goats in Sweden are *Staphylococcus aureus*, non-aureus *staphylococci* (NAS), and *Streptococci* strains, in approximately 80% of cases (Svenskagetavelsförbundet 2018b). The remaining 20% of cases are caused by environmental bacteria such as Klebsiella and *Escherichia coli* (Svenskagetavelsförbundet 2018b). Mastitis can be transmitted between lactating animals, depending on the type of bacteria (Sandholm *et al.,* 1995). One example is *S. aureus* that can be transmitted between dairy cows by the hands of the milkers (Gustavsson, 2012).

The main bacteria responsible for mastitis in camels in Kenya was investigated by Toroitich *et al.* (2017), who performed bacterial isolation from milk samples from 380 udder quarters; 114 bacteria isolates were found. The main finding was *S. aureus* with a frequency of 36.0%. The second most common finding was *E. coli* (27.2%). *Staphylococcus epidermidis* and *Streptococcus agalactiae* were found with a frequency of 9.6% each. Toroitich *et al.* (2017) claimed that the growth of mixed types of bacteria in the milk indicated a multiple infection in the sampled quarters.

An immunocompromised dairy animal is more sensitive to infections. Mastitis may occur when the lactating animal is in a sensitive stage, e.g. dairy cows are at a bigger risk for mastitis at the beginning of the dry period or the beginning of lactation (Sandholm *et al.,* 1995). Accordingly, Ahmad *et al.* (2012) found that stage of lactation and parity number had a significant relationship with mastitis also in camels and that the prevalence of mastitis was highest during the initial and last stage of lactation. Stress due to the animals´ situation could be a factor lowering resistance. Breed and lack of hygiene during milking were shown to be associated with increasing risk for mastitis in camels (Ahmad et al., 2012). In cows there is a physiological variation in SCC with stage of lactation and lactation number (Sandholm *et al.,* 1995). Obied et al. (1996) did not find any such physiological significant difference in SCC during the lactation or between lactation numbers (Table 1) in camels that were defined as free from mastitis by CMT and bacteriological examination.

Stage of lactation (months)	Lactation number 1	2	3	4	5	6	7
1-4	108 (28) [12]	120 (25·3) [18]	107 (22·1) [10]	213 (37·8) [12]	150 (21·1) [9]	212 (39·6) [11]	275 (54·9) [8]
5-8	50 (18) [28]	125 (33·1) [17]	207 (43·2) [41]	125 (26·4) [24]	200 (28·8) [21]	150 (38·4) [15]	350 (75·2) [16]
9-12	200 (48) [9]	144 (27·2) [10]	190 (34·5) [16]	144 (29·2) [8]	200 (36·6) [17]	220 (36·6) [8]	225 (48·1) [14]

Table 1: *Somatic cell counts (cell/ml) in camel milk during the lactation period and between successive lactations (Obied et al., 1996)*

An udder close to the ground could be a risk factor, due to close contact with environmental dirt and soil bacteria. Odongo *et al.* (2016) claimed that the camel´s udder is in contact with the ground while the camel is lying down. Porcionato *et al.* (2010) studied the relationship between teat morphology and the prevalence of mastitis in cows. Cows with longer teats had low-hanging udders and higher SCC. Injuries such as scratches and cuts on the udder could also start an infection that leads to mastitis (Beef and lamb 2018).

The risk factors in a dairy herd should be evaluated to prevent mastitis. Hygiene around milking and of milking equipment is essential. Healthy animals should be used as breeding stock, and provided with nutritious feed and water. If the bacteria causing mastitis in a herd are contagious, transmission can be avoided by milking animals that have low SCC before animals with a high SCC (Sandholm *et al.,* 1995). For cows, it is recommended to avoid giving female calves the milk from cows with mastitis caused by *S. aureus* (Barkema *et al.,* 2009). Keeping cows standing for half an hour after milking instead of allowing them to lie down will give the teat canal time to close before it is exposed to dirt or bacteria (Blowey *et al*., 1995). Chronic mastitis-affected dairy cows are contagious for the herd and should be culled (Sandholm *et al.,* 1995). These factors could also be applied to camel husbandry.

California mastitis test and somatic cell count

Somatic cell count increases in the udder quarter during an inflammation and is an indicator of subclinical mastitis. The SCC can be measured directly using cell counters or indirectly by the California Mastitis Test (CMT), which are ways to check the inflammatory status in lactating camels (Abdurahman, 1996). These methods are used routinely to detect udder inflammation in several dairy animals, such as cows (Jánosi *et al.,* 2004), sheep (Pradieé *et al.,* 2012), goats (Persson, 2015), and buffalo (Dhaka, 2006). The CMT test can be used easily in the field for a quick and cheap result for the pastoralists. In contrast, expensive analytical instruments are needed to be able to measure the actual cell number.

When an inflammation in the udder is triggered, commonly by an infection with microorganisms, the leukocytes (white blood cells) will increase as a defence mechanism.

This increased number of leukocytes in the milk can be measured. The CMT test is a cow-side test based on a reagent added to the bovine milk which will disrupt the cell wall, allowing the DNA and to leak out and change the viscosity in the mixture. The more cell contents that are released, the more the mixture thickens. The CMT test is highly correlated with the level of SCC in milk (Plummer *et al.,* 2012).

The increase in SCC in camel milk was shown to be similar to the increase in cattle milk during an udder inflammation. Therefore, CMT can be used to check the inflammatory status in camels (Abdurahman *et al.,* 1995). The CMT was first described by Schalm and Noorlander (1957). The scale is divided into 5 score levels, all of which are denoted by numbers (1-5) according to the Scandinavian CMT recommendations (Table 2, Goncalves, 2017). The distribution of CMT values for dromedary camels in Kenya in a study by Goncalves was as follows: 1:52%, 2:37%, 3:8% 4:3% and 5:0% of 253 camel quarters (Goncalves, 2017). The frequencies of CMT values reported by Woubit *et al*. were 1:71%, 2: 23%, 3:4%, 4:0.1% and 5:0% (Woubit *et al.,* 2001).

Scandinavian CMT – score	International Score	Criteria	SCC (cells/ml)
1	Negative	No thickening or gel formation, fluid stays homogenous	0 – 200,000
2	Trace	Mild thickening of fluid when vessel is tilted	200,000 – 400,000
3	1	Clear thickening of fluid when vessel is tilted	400,000 – 1200,000
4	2	Clear thickening of fluid with a tendency of gel formation that disappears when vessel is not rotated	1200,000 – 5000,000
5	3	Clear thickening and gel formation that remains when vessel is not rotated	>5000,000

Table 2: *International CMT scoring and the Scandinavian CMT scoring systems and their criteria. The SCC (cells/mL milk) compared to the CMT scoring are value from dairy cattle (*Goncalves, 2017).

In studies by Merle *et al.* (2007) and Bansal *et al.* (2005), cows with a SCC below 100,000 cells/ mL milk were considered to be healthy, whereas cows with SCC higher than 100,000 cells/ mL milk in one of the udder quarters were considered to have inflammation. Hamed *et al.* (2010) compared low and high SCC between cow and camel milk. Two groups were created, one with SCC ≤ 105 cells/mL and the other one with SCC ≥ 105 cells/mL. Camel milk had a lower mean value of SCC in both the high and low SCC groups. The SCC for camels in comparison with the cows in the lower scoring group was 25.5±16.4 $x10^3$ and 32.5±23.9$x10^3$, respectively. In the high scoring group, the SCC for camels and cows were 331.4±436.7$x10^3$ and 369.1±433.2$x10^3$, respectively.

Many countries use SCC as an indicator of milk quality, with the farmer receiving either a reduction or an increase in payment for the milk based on the results. Countries that do not have milk payment based on milk quality often have poorer milk hygiene (Pasic *et al.,* 2016).

The biggest dairy in Sweden, Arla, has a 2% increase in commodity value if the SCC in the bulk milk is lower than 200,000 cells/mL milk. However, a level above 300,000 cells/ mL milk will result in a reduction in payment, which can be up to 10% if the SCC shows more than 400,000 cells/mL (Arla.se 2019).

A wide range of SCC in camel milk has been reported. In a study done by Merin *et al.* (2004) the mean SCC of milk from healthy camel udders was 118,000 cells/mL, and an udder with inflammation had a mean SCC of 308,000 cells/mL milk. Abduraham (1995b) reported that an average SCC in camel milk for quarter with no growth of bacteria was between 216,000 cells/mL and 415,000 cells/mL; camel udders with quarter milk SCC above 550,000 cells/mL should be considered to be infected.

In the early 1900s SCC was counted manually using the direct microscopic somatic cell count (DMSCC). This method is commonly used as a reference and was described first by Prescott & Breed. (1910). A small amount of milk is spread over a surface where it will dry, allowing the cells to be stained for observation and counting using a microscope. This method is still being used nowadays with some modifications (Gonçalves *et al.,* 2018, Abdurahman *et al.,* 1996). However, this technique is time-consuming and results can vary between different observers depending on how they interpret what they see in the microscope (Gonçalves *et al.,* 2018).

New techniques have been developed to make cell counting easier and faster, and to reduce differences in interpretation. The new Fossomatic 7 (FOSS) can count up to 600 milk samples in one hour. The FOSS technique uses flow cytometry where the milk cells are run through a capillary pipe and counted by photo electronics. The precursor to Fossomatic 7 was first developed in 1980s. The Fossomatic technique is used in milk testing centers (Fossanalytics, 2018).

DeLaval has developed an automatic optical cell counter (direct cell counter [DCC]), that is also portable. A picture is taken with a digital camera of the nuclei of the somatic cells which have been stained with fluorescent reagent and these are then counted individually. The count is displayed after 45 seconds (DeLaval 2003).

When DCC was compared with DMSCC on a large-scale camel dairy farm, there was a strong correlation between the cell counting measurements. The mean DCC (363,000 cells/mL milk) was slightly lower than for DMSCC 398,000 cells/mL milk. The two different methods had the same coefficient of variation of 23.5*%. (*Nagy *et al.,* 2013*).*

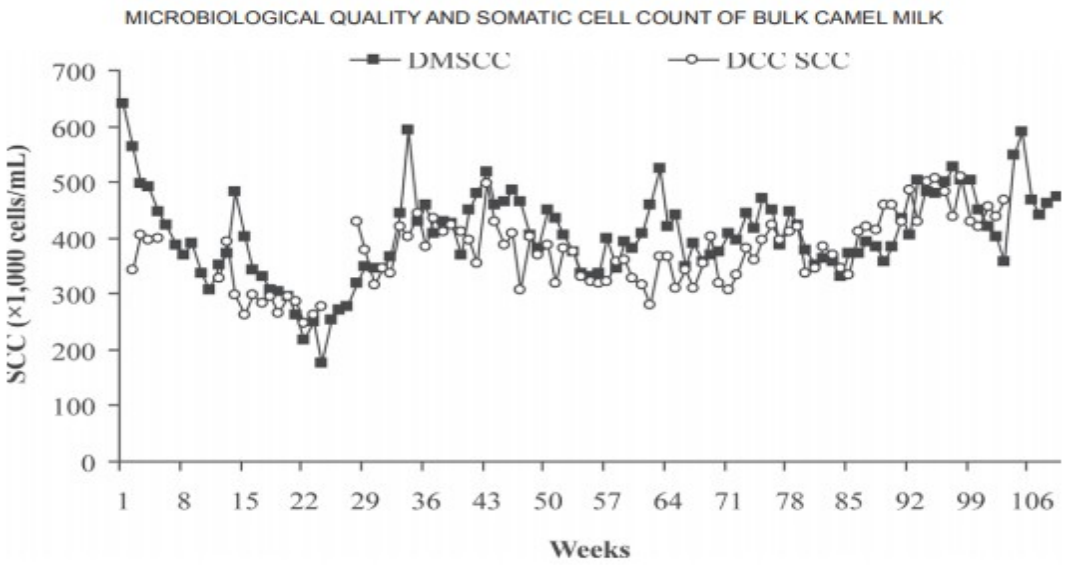

Figure 1: *The change in mean SCC per week for 2 years using the SCC methods DMSCC and DCC (Nagy et al., 2013).*

Relationship between mastitis and milk yield

The milk yield of camels was investigated in several studies (Zelek. 2007: Onjoro *et al.,* 2006; Bekele *et al.,* 2002) and shown to vary naturally during the lactation period. Other factors affecting the milk yield include the accessibility of feed and water, dry or wet season, age, and SCC (Zelek, 2007).

The camel's udder differs from that of other dairy animals, mainly in the ability to store milk. The camel stores about 90% of the total milk yield in the alveoli (Ayadi *et al.,* 2013*),* while many other dairy animals store a large amount in the milk cistern. Goats can store up to 75% in the cistern, cows 30%, and dairy sheep 50% (Costa *et al.,* 2003). When more milk is kept in the alveoli, a strong milk ejection reflex is necessary for complete emptying of the udder (Atigui *et al.,* 2016). Cows, goats and sheep have one teat canal attached to the udder cistern. The camel has two, sometimes even three, teat canals, each attached to a separate glandular complex, whereas horses and pigs have 2-3 teat canals each leading from a separate udder cistern (Husvéth, 2011)

In a report by Onjoro *et al*. (2006), the mean daily milk yield was 3.4±0.2 L/d (n=12) for free-ranging camels in northern Kenya at Kenya Agricultural Research Institute (KARI) field station. The milk yield increased from 3.4 L/d in the dry season to 4.3±0.3 L/d in the wet season. The recordings that KARI made on its own camels corresponded to a mean value at 3.2 L/d with Onjoro *et al.* (2006). The mean milk yield recorded for Somali camels in Ethiopia was reported to be 4.14±0.04 kg/day (n=61) (Bekele *et al.,* 2002), which is in agreement with the KARI and Onjoro *et al*. (2006) studies on milk yields for camels. Bekele *et al.* (2002) also reported that the daily milk yield in camels can be enhanced by increasing the number of milkings. With one milking per day, a production of 1.26±0.05 kg was recorded, whereas with four milkings per day the daily yield increased to 6.77±0.15 kg.

In a literature review by Hortet *et al.* (1998), data collected from 20 studies on milk yield losses and composition changes due to clinical mastitis in dairy cows were analyzed. Eight of the studies compared milk yield from cows with mastitis against the cows´ own milk yield

from a previous lactation. They also compared it with cows in the same herd that were considered healthy and had never been treated for mastitis. From these eight studies, five looked at the milk yield loss without considering the lactation number. The average losses due to mastitis varied from 0% to 9.5%. In nine other studies that took into account the cows´ lactation number, milk yield losses varied between 0.5% and 6.4%. Lucey *et al.* (1984) reported a milk yield loss of 11% when comparing the milk yield before the peak of the lactation curve with the whole lactation, as well as a 6.4% milk yield loss for cows with mastitis compared to those without mastitis. This type of study has not been performed on camels.

One reason why the milk yield decreases during mastitis is that when an udder inflammation occurs, the udder epithelial cells will be damaged. That means that the synthesis of lactose, fat and protein will be reduced and the milk will contain less of these substances (Sharif *et al.,* 2007) The reduction of lactose, which is the major osmosis-regulating substance in milk, results in a reduced amount of milk (Deluyker, 1991).

Forster *et al.* (2010) studied milk yield and CMT in individual quarters of dairy cows and compared a mastitic quarter with the opposite quarter with a negative CMT score. The study used measurements of one milking from 763 cows. The results were distributed between both front and rear udder quarters and throughout the whole lactation period. Udder quarters with CMT scoring of "trace, 1, 2 and 3" were shown to be related to a decrease in milk yield as follows: CMT trace, 9.0%; CMT 1, 19.5%; CMT 2, 31.8%; and CMT 3, 43.4%. Merle *et al*. (2007) and Bansal *et al.* (2005) reported that if a cow had one udder quarter with an infection, the other healthy udder quarter would also have a higher level of somatic cells than a quarter from an udder that is completely free from infection. In contrast, Barkema *et al.* (1997) stated that an intramammary infection (IMI) is often restricted to one udder quarter and does not spread to the other quarters by itself. However, they considered that transmission of contagious bacteria such as *Str. agalactiae* and *S. aureus* by milking equipment or between cows to be possible.

A severe case of mastitis or an unhealed teat injury could result in cessation of milk production from this quarter (Jones, 2009). Compensatory changes in the milk yield between cow udder quarters that were milked or not milked were investigated by Hamann *et al.* (1990). The average daily milk yield was measured in a pretreatment period for each cow included in the study; then one, two or three udder quarters were selected for non-milking. After the 12 days treatment period, another period of 12 days was initiated when the cows were milked from all four quarters again. The milk yield during the 12 days´ treatment period for the cows that were milked continuously from one, two and three quarters was increased by ~14%, ~10% and ~4% of the mean daily milk yield in the treatment period. The udders that were milked from one quarter produced 78% of their original average daily milk yield after the treatment period was over (Hamann *et al.,* 1990).

Enzymes NAGase and LDH

The enzyme NAGase is a lysosomal enzyme that is released if somatic cells, such as epithelial cells, are damaged and the cell content and plasma proteins leak out (Kitchen *et al.,* 1980, 1978). Another enzyme, LDH, is released during lysis of mammary cells (Singh *et al.,* 2015). During an infection in cows, the levels of NAGase and LDH will be much higher than in a healthy cow due to damaged cells (Chagunda *et al.,* 2006). The NAGase activity has also

been used as an inflammation indicator for ewes (Maisi *et al.,* 1987) and for goats (Timms *et al.,* 1985), and LDH- activity was described as a good indicator of subclinical mastitis in buffaloes (Singh *et al.,* 2016).

Hovinen *et al.* (2016) stated that both subclinical mastitis and clinical mastitis can be detected by NAGase with an accuracy of 85% and 99% in dairy cows. They also showed that the level of NAGase increases when the SCC increased. In the first 30 days of the cow´s lactation the NAGase activity was higher than in the rest of the lactation. Hovinen *et al.* (2016) also found that NAGase was higher in milk from older cows than from younger cows. This corresponded with findings in a report by Nyman *et al*. (2014) in which both the milk enzymes, NAGase and LDH, were higher in older cows than younger ones, and higher in mastitic cows than in those without IMI. The values for NAGase in IMI negative cows varied between 0.02-15.6 U/L whereas the IMI positive values varied between 1.28-15.3 U/L. The values for LDH in IMI negative cows varied between 0.15-12.0 U/L whereas the IMI positive values varied between 0.48-20.4 U/L (Nyman *et al.,* 2014). In a study by Åkerstedt *et al.* (2010), the NAGase and LDH in clinical healthy cows was 0.8-6.1 U/L and 1.1-3.2 U/L, resepectively, whereas the cows with subclinical mastitis had mean levels of 25.0 ± 28.9 U/L for NAGase and 45.0-58.9 U/L for LDH. Hovinen *et al.* (2016) found no differences in the NAGase activity between seasons. However, Nyman *et al.* (2014) reported that both LDH and NAGase activity were significant lower from September to November compared with December to April.

Leitner *et al.* (2004) studied the relationship between bacterial status and NAGase activity in 10 dairy goat herds. Bacterial status had a significant effect on NAGase activity. Barth *et al.* (2010) showed a similar relationship between NAGase activity and the infection status of goat milk samples. In contrast, Guliye *et al.* (2002) found that in camels there was no difference in NAGase activity in quarter milk samples that contained bacteria compared to those that did not. The type of bacteria in the udder did not influence NAGase activity (Guliye *et al.,* 2002). However, the differences in the NAGase and LDH activities in the study on bovine milk could depend on whether sampling was done from quarter milk or from composite milk (Hovine *et al.,* 2016)

The LDH and NAGase activities were more affected by cow factors such as days in milk, milk fat % and protein %, urea concentration, breed and milk yield, than was the SCC (Nyman *et al.,* 2014). The IMI status accounted for 23% of the variation in the SCC measurements whereas they explained only 7% and 2%, respectively, of the variation in NAGase and LDH (Nyman *et al.,* 2014). A high increase in SCC, NAGase and LDH in the monthly test milking results may be the result of an inflammatory response because of IMI instead of cow factors such as parity and days in lactation. Nyman *et al.* (2016) concluded that SCC was generally the most efficient way of identifying IMI-positive and IMI-negative dairy cows.

NAGase activity is also found in the blood (serum and in white blood cells); this enzyme can pass through the blood-milk barrier and be measured as NAGase from the epithelial cell cytoplasm of the mammary glands (Nagahata *et al.*, 1987; Kitchen *et al.,*1978). The NAGase enzymes that were obtained from the blood were reported by Kitchen *et al.* (1978) to be 5-15% of the total NAGase activity in cow milk. Piccinini *et al.* (2005) showed that the same amount of NAGase activity was observed in blood samples from both healthy dairy cows and dairy cows with a positive IMI status. However, the NAGase levels in quarter milk were significantly higher in unhealthy cows than healthy ones.

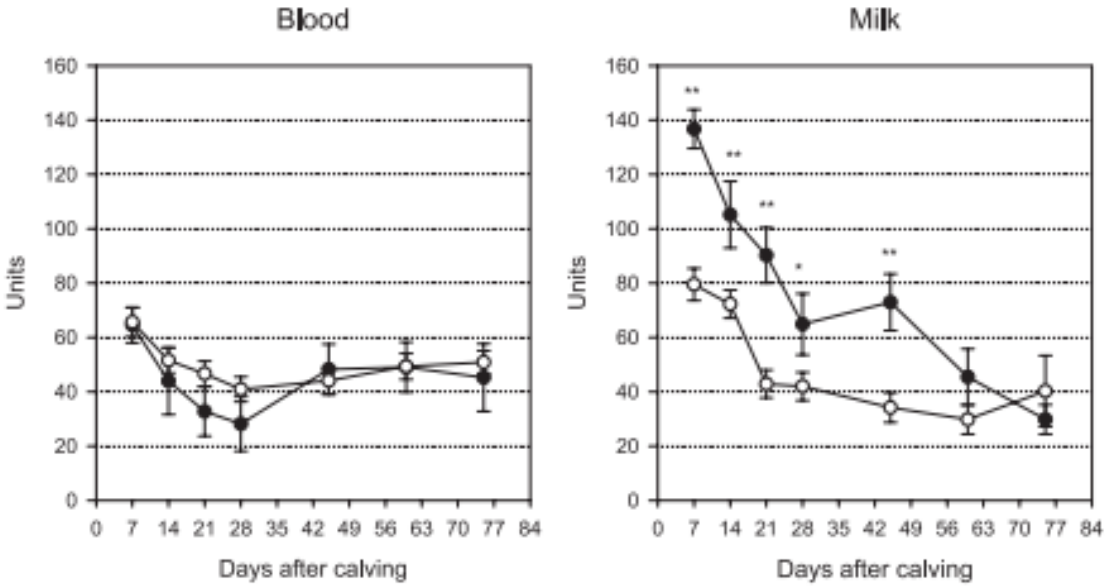

Figure 2: Distribution of NAGase in blood and milk from healthy (white) and IMI status (black) dairy cows (Piccinini et al., 2005).

Camel milk contains a high proportion of cell fragments without a nucleus. Abdurahman *et al.* (1992) compared camel milk, which contains a high number of this cell fragments, with goat milk, which also has a large amount of broken cell fragments in the milk. These cytoplasmic particles are a similar size to epithelial cells and could be mis-read as a high cell number. To avoid this error, a DNA-specific method should be used, such as a technique that counts cell nuclei (Nagy *et al.,* 2013). Abdurahman *et al.* (1992) considered that this could be a reason why camel milk has a higher NAGase activity than, for example, cow milk. A high milk yield of cows was shown to be associated with low LDH and NAGase activity (Nyman *et al.*, 2014).

Bacteria associated with mastitis

The type of bacteria infecting the udder of the camel will affect the SCC (Guliye *et al.,* 2002). The main mastitis bacteria for camels in Kenya are *S. aureus, E. coli, S. epidermidis* and *Str. agalactiae* (Toroitich *et al.,* 2017). Guliye *et al.* (2002) found that the SCC was highest if the udder quarter was infected with *S. aureus* and lowest if the infection was caused by *E. coli.* Although *S. aureus* are able to grow successfully between 8°C-46°C, the optimal growing temperature is 38.5°C (Medvedova *et al., 2*009).

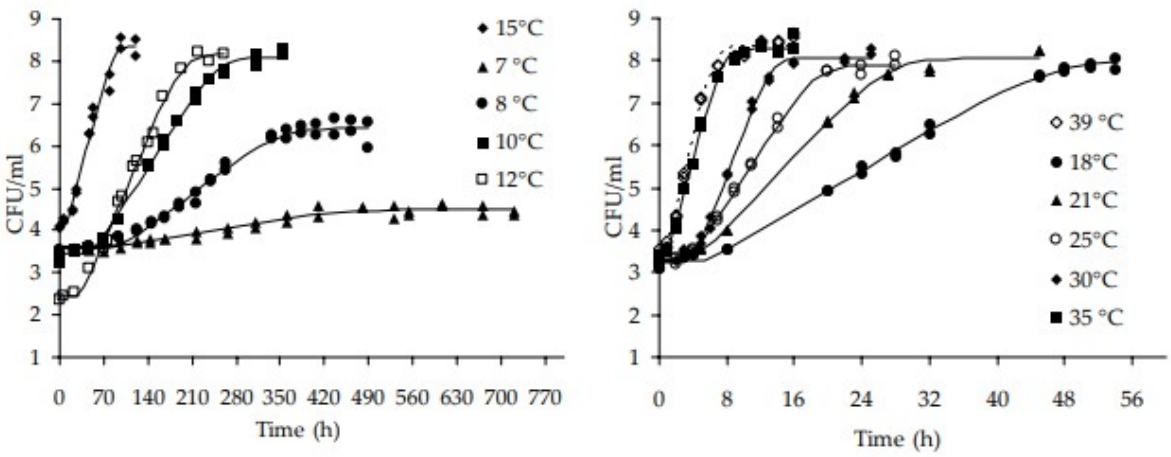

Figure 5: *The growth of Staphylococcus aureus 2064 in human milk at various incubation temperatures (Medvedova et al., 2009).*

Staphylococcus aureus is a highly contagious bacterium that is transmitted to other animals within the herd via milking equipment and the milkers´ hands; *S. aureus* could also infect the calf through the cow´s milk (Radostits *et al.,* 2007). In herds that have severe problems with the bacterium, *S. aureus* can also be found in wounds and on the skin of the hocks (Gustavsson, 2012). If there are wounds on the teats from cuts or from the cow stepping on the teat, the risk of *S. aureus* infection will be 93% compared with an undamaged teat where the risk for infection would be 53%. When a cow has *S. aureus* in its body, it is difficult to remove, even with treatment. The infections are often chronic and *S. aureus* is able to survive intracellularly (Radostits *et al.,* 2007).

Treatment

After a dairy cow with mastitis has been treated with antibiotics, the recovery period could last several weeks, as seen in a reduced milk yield and in LDH activity levels. Cows that had already been treated once for *S. aureus* mastitis had a 16% lower milk yield than control cows (Fogsgaard *et al*., 2015).

It is possible to treat mastitis bacteria, but the results vary. Younan (2002) reported that camel herdsmen were familiar with injectable drugs as a method of administering antibiotics, whereas they were not familiar with intramammary tubes. The teat canals of camels have a smaller dimension than those of cattle, which makes intramammary tubes unsuitable for camels. Intramammary treatment can cure up to 87.5% of *Str. agalactiae* mastitis infection in dairy cows if the cases were detected early, while the cure rate is reduced to 14.7% by a late detection and treatment (Hejlicek *et al.,* 1994). Younan, (2002) stated that the pastoralists camel keepers usually “only consider treatment” when mastitis is acute.

The conclusion from Younan (2002) is that the types of antibiotics and the dose rates used in the treatment of dairy cows could work well for camels. However, because of strong sunlight and high temperatures in the camel's living conditions, the stability of the drugs may be questionable and should be further investigated.

Temperature as an indicator of mastitis

The possibility of detecting mastitis early in dairy cows using a thermal camera has been investigated in several studies (Sathiyabarath *et al.,* 2016; Polat *et al.,* 2010; Hovinen *et al.,* 2008, Berry *et al.,* 2003). The advantage of using an infrared thermal camera is that it is a non-touch method and is rapid (Berry *et al.,* 2003).

Hovinen *et al.* (2008) introduced *E. coli* lipopolysaccharide into the left front udder quarters of six cows, while the right front udder quarters served as control quarters. The thermal camera could detect the induced mastitis by showing a 1-1.5°C increase in udder temperature. However, the clinical signs such as swelling and changes in the milk were seen before the temperature increased. Thus, their study did not support the theory of detecting mastitis at an early stage with a thermal camera (Hovinen *et al.,* 2008). In contrast, Scott *et al.* (2000) found that the udder surface temperature increased by 2.3°C when mastitis was induced with bacterial endotoxin.

The natural variation in udder temperature for dairy cows was investigated by Barry *et al.* (2003). Environmental temperature, together with the previous day´s udder temperatures, could together predict the expected udder temperature with a high precision. With this knowledge a baseline of expected udder temperature could be created. A difference in the expected udder temperature could indicate mastitis in the early stages. They also showed that the cow's udder temperature rose with exercise outside (Barry *et al.,* 2003).

The body temperature of a camel can have a 6°C variation, which is part of the camel´s way of conserving water (Wilson, 1998). Sathiyabarath *et al.* (2016) measured the body temperature of dairy cattle for 28 days, which was found to be 37.23 ± 0.08 °C, and the average udder skin surface temperature was 37.22 ± 0.04 °C. The cattle that were classified as having subclinical or clinical mastitis by CMT had an increase in udder temperature of 0.72 and 1.05°C, respectively. In another study, it was seen that the temperature of the cows´ udder surface was starting to increase up to 3 days before the onset of any clinical signs of mastitis (Hurnik *et al.,* 1984).

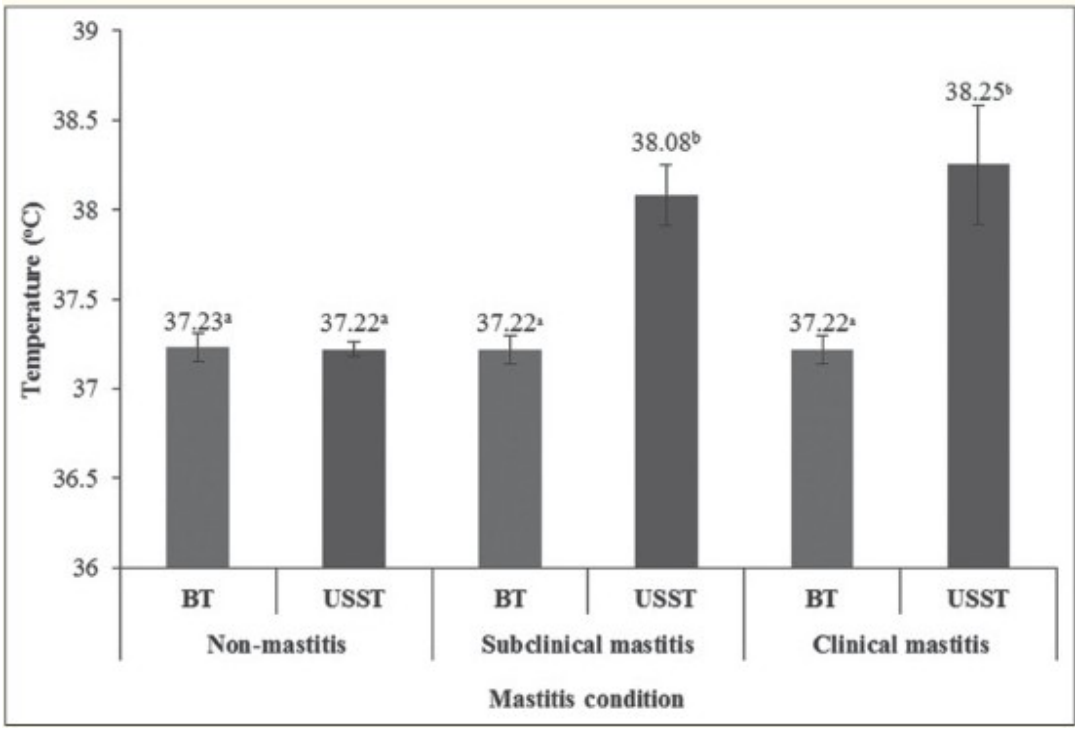

Figure 6*: The differences in udder skin surface temperature (USST) and body temperatures (BT) between non mastitis, subclinical mastitis and clinical mastitis in Holstein Friesian dairy cows (Sathiyabarathi et al., 2016)*

Apart from dairy cows, the technique of measuring udder surface temperature has been tested on dairy goats (Caruolo *et al.,* 1990) and sheep (Castro-Costa *et al.,* 2013; Mala *et al.,* 2009). Samara *et al.* (2013) studied the possibility of using an infrared thermometer on machine-milked dairy camels (*Camelus dromedarius*). The SCC and CMT were used for detection of subclinical mastitis. According to the CMT and SCC, the udder quarters with subclinical mastitis had a 1.42°C higher temperature than healthy quarters. Thus, they concluded that infrared thermography could be a method for early detection of subclinical mastitis (Samara *et al.,* 2013).

In a study by Polat *et al.* (2010), a correlation between CMT and udder skin temperature in dairy cows was observed. Negative CMT had a SCC mean value of 65 $x10^3$ cells/mL and a mean udder skin temperature of 33.23 °C, whereas a CMT of 3+ had a mean SCC of 3,653 $x10^3$ cells/mL and a mean udder skin temperature of 36.27°C. In their conclusion, they suggested that further research is needed to investigate how various cow factors, such as lactation month, age, milk production and feeding times, as well as environmental factors such as humidity and temperature, could interfere with a reliable infrared thermometer reading.

Materials and Method

Study area and herds

The study was conducted in the beginning of January to the end of February 2018, in the Laikipia district of Kenya. The study included six pastoralist camel (*Camelus dromedarius*) herds. The herds were chosen after consideration of the following: being similar holdings with similar feed and environments, the ease of access from the researchers´ basecamp, and the interest from the owners of the herds to participate in the study. The camels were kept under pastoralist management in a semi-arid area where feeding consisted of natural browsing. Due to time limitations and collaboration with camels and herdsmen, four of the six herds (A, B, C and D) were chosen for repeated measurements.

Picture 1: Maps of the locations of the sample area and the locations of the studied herds.

All the camels were held in traditional enclosures, or "bomas", overnight and at milking, whereas during the daytime they were herded in the surrounding area. The calves were separated from the group only at night, being allowed free access to their dams during the day. The milking frequency was 1-2 times a day at the time of sampling which was during the dry season.

No feed supplementation other than minerals and salt was added by the herdsmen. Most of the camels were of Somali breed with a few exceptions of Pakistan and Turkana breeds. The number of camels included in the study was 97, comprising 10 camels in Herd A, 40 in herd B, 11 in herd C, 20 in herd D, 6 in herd E and 10 in herd F. Herds A, B, D and F were watered once every second day, while herds C and E were watered once a week. In all the herds, the camels were treated against ticks.

Sample collection

The sample collection were made over a six week period. Herd visits were performed 30 times, varying from 3-7 herd visits per week. The visits were performed depending on the possibility of the herd to host the researchers and the accessibility of transportation. The visits were divided as equally as possible between the herds during the six weeks. The duration of the visit was between 1.5 and 2.5 hours.

The camels were examined visually to confirm that their general health was good. Behavioural signs, such as normal activity, normal movement pattern, and showing curiosity, were noted as indicators of health.

California mastitis test

A visual inspection was made first to look for any signs of swelling, redness or injuries on the udder or abnormalities in the colour or texture of the milk that could indicate clinical mastitis. A CMT-test (Scandinavian scoring) was conducted at the first meeting with the camel herds at the camel boma, except for herds D and E, where the milk was collected in 10mL milk tubes and transported in a cooler to the lab at Mpala research center, where the CMT test was conducted 2h after milking. The herdsmen released a camel calf from the enclosure where the calves were kept during the night. Milk letdown was initiated by the stimulation of the calf's presence and attempting to suck. The final udder stimulation was done manually by the herdsman. When the milker felt that the milk had been let down in the udder cistern by the camel, one strike of milk was pulled out on the ground. Then the CMT paddle was filled with milk from one or two strikes, which was mixed with CMT liquid and stirred, and the result recorded.

Herd A had the smallest number of positive results of subclinical mastitis and was chosen as a control herd for milk percentage differences between paired udder quarters. The camels in herds B, C, and D that showed a positive CMT score were chosen to continue in the study if they had an opposite udder quarter that had a negative CMT test. In this way, the camels could serve as their own controls but could also be compared with the healthiest herd.

The results from the test were used to distinguish the camels with positive CMT readings (score ≥2) from the ones with negative CMT reaction (CMT score 1/ healthy). From these results, the camels that had quarter pairs (either front or hind) with one quarter with positive and the other quarter with negative CMT reaction, were chosen for continued sampling in the repeated measures study.

Picture 2: Camel milk mixture with CMT- contrast liquid on a CMT-paddle, right bottom corner indicates a strong positive reaction.

Somatic cell count

The SCC was analysed using Delaval´s Somatic Cell Counter (DCC). For herds A, B, and C, the milk from the CMT positive quarters and healthy opposite quarters was analysed the day after the CMT was performed due to time limitation. For herd D, the SCC was analysed the same day as the CMT was conducted.

The milk was collected in 10ml milk tubes during milking, when the milkers indicated that the camel had started to let the milk down. After milking, the milk was transported in a cooler to the lab at Mpala Research center. For camels that had produced milk samples showing a positive CMT reaction in one quarter and a negative CMT reaction in the opposite quarter, the samples were read using the DCC. The SCC was analysed for 179 milk samples.

Picture 3: The Delaval´s somatic cell counter DCC and the cassette used to draw the milk up in capillary tubes.

NAGase and LDH

N-acetyl-beta-D-glucosaminidase and Lactate dehydrogenase were analysed in the same samples as collected for the DCC. A total of 144 milk samples was frozen at -20°C in 4ml white bronopol tubes. The tubes were transported on dry ice from Nairobi, Kenya at the end of the study to Foulum Research Station Viborg, Denmark, where the enzymes levels were measured. NAGase activity was specified with an endpoint fluorometric assay according to Larsen (2010). LDH activity was analyzed with a fluorometric kinetic method according to Larsen (2010).

Milk yield

The milk measuring equipment was made from a 10 litre bucket with a lid. Inside the bucket, 4 x 1 litre plastic bottles were placed in mugs to stabilize them. The lid of the bucket had 4

openings in the top through which the top of the bottles protruded. Above the lid in the 4 bottle openings, 4 funnels were joined together. Using this construction, each udder quarter could be milked into separate bottles. Each bottle had a string tied around its neck. The string was used to hang the bottle on a scale to measure the milk in the bottle. The scale had an accuracy of two decimal places.

The bottle and string weighed together 0.03kg which was deducted from the total weight of the bottle and milk to give the milk yield measurement. The different colours on the funnels matched the string colour around the bottle necks to enable the milkers to keep track of which udder quarters had been sampled, to ensure that milk yield was measured and recorded correctly. After a bottle had been weighed, the contents were emptied into another container for the herdsmen to handle.

Picture 4: The milk bucket, allowing milk from each quarter to be collected separately for measurement of quarter milk yield.

Picture 5: The separate containers that contained the milk yield from the four udder quarters were weighed with a digital "hock" scale.

Temperature

The udder skin surface per quarter was measured repeatedly in herds A, B, C and D. The outside temperature was measured in the mornings on arrival and departure from the herds.

The temperature measurements were performed just before the calf started to stimulate the camel udder by sucking, just before the stimulus from the milkers started. The thermometer was a "Microlife NC150 non-contact thermometer", which used infrared light to measure the udder skin temperature. The thermometer was held at a distance of 3 cm from the udder surface skin. The temperature was shown for 3-4 seconds. The thermometer had a built-in memory for up to 30 temperature measurements. The camel´s four udder quarters were measured in a sequence. The temperature measurements were taken at the same milking as the milk yield was recorded, while the camel was milked. The thermometer was new and not calibrated during the study. Due to time limitations, the temperature was not measured the same day as the CMT.

Picture 6: The thermometer was held at a distance of 3 cm from the udder surface skin. "Microlife NC150 non-contact thermometer", (Picture Apotek Kronan)

Questionnaire

A questionnaire was created with three questions for the herdsman most responsible for the herd. The answers were recorded for 57 camels.

1. The number of calves the camel had produced
2. The lactation month of the camel
3. If the herdsman thought the camel produced a high, medium or low amount of milk

The answers for the third question were noted down as low = 1, medium = 2, high = 3.
The three answers were then compared with the camel´s highest CMT value from the four quarters.

The milking routines in the herds included in the study were observed for milking times, milking order, hygiene, equipment, storage and transport. Also the herdsmen were questioned about mastitis and subclinical mastitis, and their knowledge about the subject. Traditions and habits were discussed through conversations with the herdsmen and participants.

Statistical analyses

Descriptive statistics of distributions of SCC, NAGase, LDH and milk yield over CMT and all statistical analyses were performed in Stata (Release 15.1; College Station, TX, USA: StataCorp LP). Associations between CMT and SCC, NAGase, LDH and milk yield, as well as between SCC and milk yield, SCC and quarter placement, SCC and NAGase and SCC and LDH, were investigated using linear mixed effect regression models adjusting for repeated measurements within camel and herd.

Nagase and LDH values were compared with both CMT score and SCC from the same udder quarter.

Comparisons between milk yield in matched udder quarters were made using Wilcoxon-Sign

Rank Test. The quarter milk yield measurements taken in the 10-12 day period were then compared with the CMT result the camel had shown at the first CMT measurements. The milk yield measurements between CMT 1, 3, 4 and 5 were compared with CMT 1. The milk yield difference was then calculated in percentage. First, the CMT 1 udder quarters were compared with each other, to show the milk yield difference in percentage between two healthy udder quarters (CMT1), either “front front” or “hind hind”. Second, the milk yield from the quarter that was defined as healthy was compared with the milk yield from the other unhealthy quarters. The difference in milk yield from the compared udder quarters was calculated (%).- The differences in milk yield between quarters with CMT 1 and quarters with CMT 3, 4 and 5 were calculated to see how much the milk yield differs between udder quarters where one udder quarter is affected with subclinical mastitis. The results are shown in Figure 6.

An investigation was carried out to determine if the number of parities affected the subclinical mastitis status of the dam. A comparison between CMT and the lactation month was performed to investigate if CMT had any association with early, mid or late stage of lactation. The lactation month was compared with the highest CMT score of the camel´s four quarters. A comparison between CMT and the herdsman´s assessment of milk yield performance was done to see if the CMT had any association with the evaluated production level. The answering options for the herdsmen were low producer, middle producer or good producer, with the answers being given a number: low producer = 1, middle producer = 2, good producer = 3.

Results

Subclinical mastitis indicators and milk yield

CMT

In all, milk samples from 505 udder quarters from 97 camels in 6 herds were investigated using CMT; the results are presented in table 1. The median CMT score was 1 (inter quartile range (IQR) 1 – 1). There was at least one camel in each herd that had a quarter with CMT≥3. The herd prevalence of camels with CMT≥3 for the six herds A, B, C, D, E and F was 10%, 14.7%, 20.9%, 22,5%, 37.5% and 35.3%. The prevalence of CMT ≥3 on udder quarter level was 2.5%, 7.1%, 14%, 17.5%, 17.6% and 25%.

Table 1: Distribution of Californian mastitis test scores (number of quarter milk samples (%) and distribution, median (inter quartile range (IQR)) and mean values (standard deviation (SD) of (SCC, NAGase, LDH and milk yield) in quarter milk samples and quarter milk yield for each CMT score.

CMT score	1	2	3	4	5
n	389 (77%)	47 (9%)	29 (6%)	25 (5%)	15 (3%)
SCC,*1000cells/ml					
n	72	22	21	17	12
Median (IQR)	48.5 (23.5 – 112)	206 (126 – 470)	434 (331 – 888)	1370 (923 – 1998)	4561 (3929 – 5551)
Mean (SD)	234 (820)	303 (227)	702 (668)	1627 (1180)	4501 (1148)
NAGase					
n	58	14	17	13	7
Median (IQR)	16.5 (13.6 – 21.2)	20.6 (17.0 – 27.7)	19.2 (16.0 – 23.6)	30.4 (21.7 – 45.9)	42.1 (29.5 – 111.4)
Mean (SD)	18.5 (8.1)	21.8 (6.4)	24.6 (21.5)	38.4 (23.2)	59.5 (43.0)
LDH					
n	58	14	17	13	7
Median (IQR)	8.8 (7.2 – 12.6)	12.6 (10.5 – 14.6)	12.7 (10.6 – 16.4)	25.7 (21.1 – 33.3)	64.6 (35.1 – 81.5)
Mean (SD)	10.4 (5.3)	14.2 (6.7)	14.2 (6.7)	29.5 (16.0)	62.3 (23.1)
Milk yield					
n (not including milk yield=0.01)	60	9	9	6	2
Median (IQR)	0.33 (0.02 – 0.42)	0.24 (0.22 – 0.31)	0.32 (0.12 – 0.38)	0.15 (0.14 – 0.17)	0.11 (0.06 – 0.17)
Mean (SD)	0.33 (0.15)	0.25 (0.06)	0.31 (0.19)	0.19 (0.14)	0.11 (0.08)

Somatic cell count and association with CMT

The SCC was analyzed in 144 udder quarter milk samples. The median SCC was 162,000 cells/mL (IQR: 48,500- 888,500 cells/mL); the mean SCC was 832,800 cells/mL (SD:1446 200 cells/mL). There was a linear association between SCC and CMT where SCC was significantly higher with increasing CMT for all comparisons of CMT classes ($p<0.01$) (Figure 1).

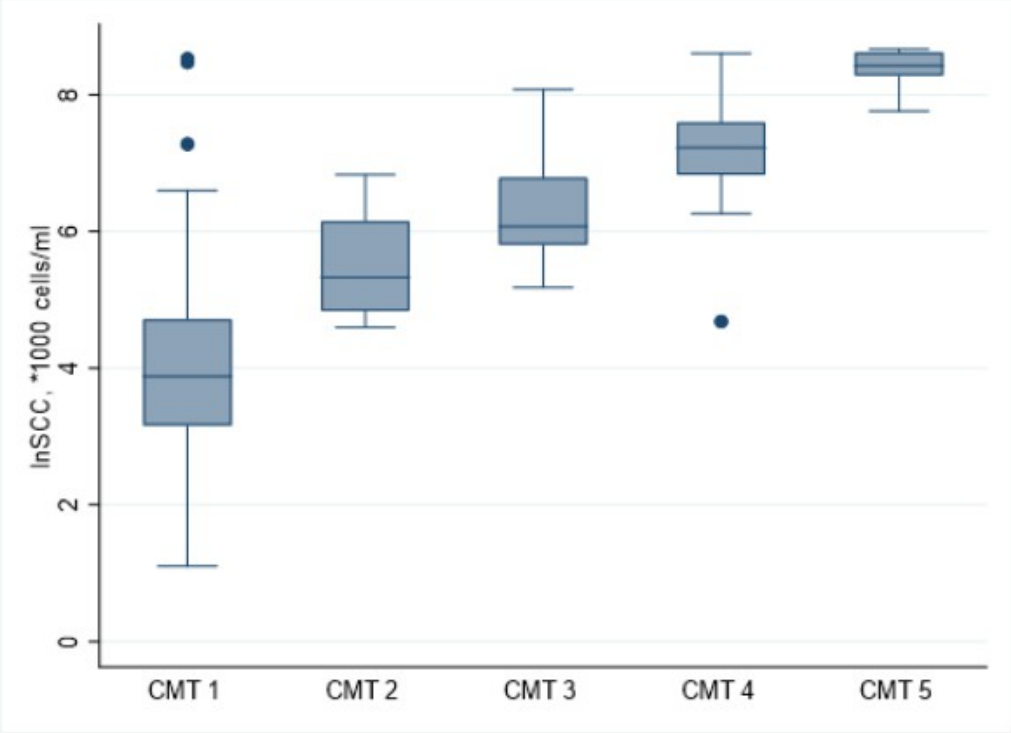

Figure 1: Box-and-whisker plot of somatic cell count (log converted ic to lnSCC) levels in milk samples with CMT scores 1-5.

A significant association was seen between the udder quarter placement and SCC, being lowest in the right front quarter and highest in the right hind quarter.

Table 2: The relationship between the position of udders quarters and SCC.

Teat position	Observation	Mean (SD)	Min	Max
LF	40	997 (1527)	26	5461
LH	33	700 (1307)	10	5549
RF	39	623 (1242)	4	5784
RH	32	1021 (1708)	3	5561

NAGase and LDH and associations with CMT

The enzymes NAGase and LDH were analysed in 111 samples. The median and mean NAGase activity was 19.6 U/L (IQR:15.3 – 26.3) and 24.9±19.8, respectively. The median and mean activity of LDH was 12.0 U/L(IQR: 8.5 – 16.8) and 17.1±16.1, respectively. There was a significant association between the NAGase levels and CMT with significantly higher NAGase levels in milk with CMT 4 and 5 compared to milk with lower CMT scores (p<0.05) Figure 2. There was also a significant association between LDH levels and CMT with significant lower LDH levels in milk with CMT 1 compared to all other CMT categories (p<0.001) and when comparing all other CMT categories with each other (p<0.001), except for CMT 2 compared with CMT 3 (p=0.59) (Figure 3)

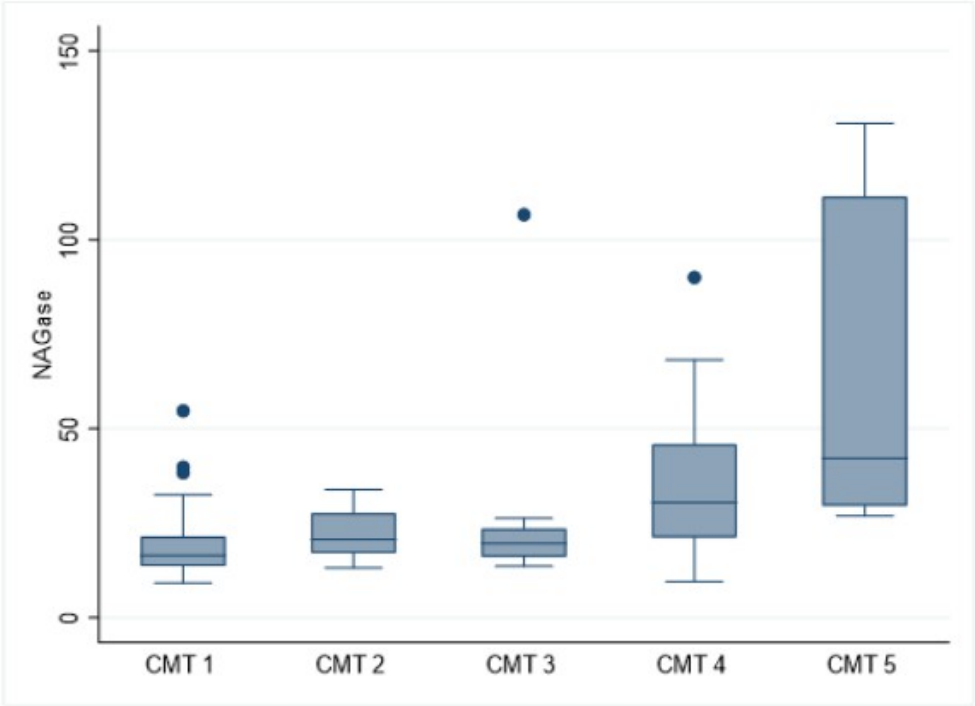

Figure 2: Box-and-whisker plots of N-acetyl-beta-D-glucosaminidase (NAGase) enzyme levels for quarter milk samples with CMT scores 1 – 5.

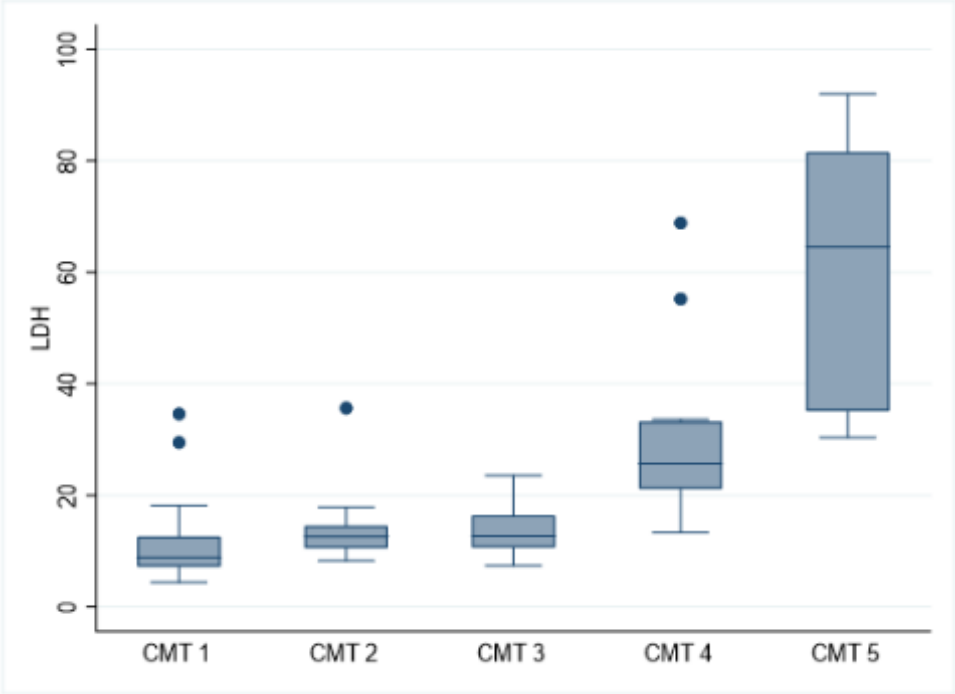

Figure 3: Box-and-whisker plots of Lactate dehydrogenase (LDH) enzyme levels in milk samples with CMT scores 1 – 5.

There was also a significant relationship between NAGase and LDH levels and SCC. (Figure 4), where the NAGase and LDH levels were higher with increasing cell count.

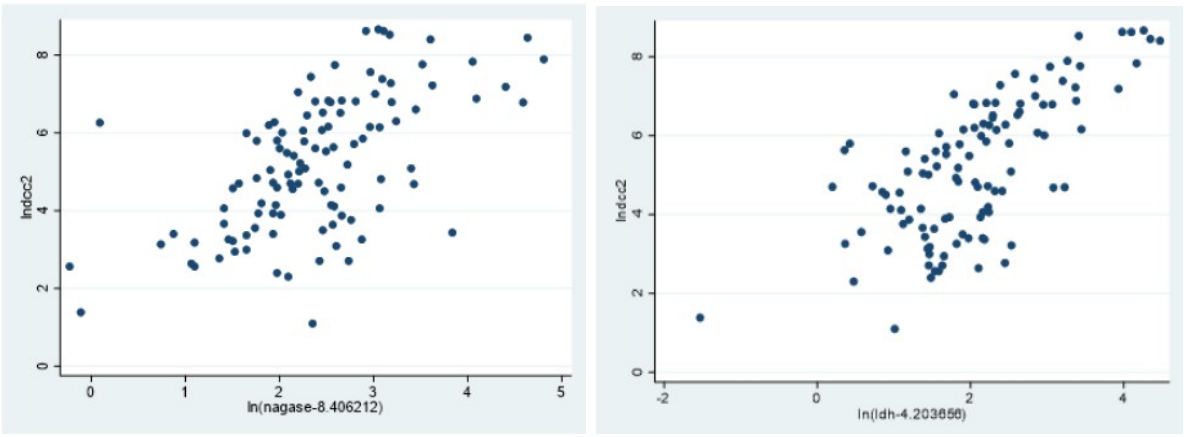

Figure 4: Relationship between SCC and enzymes NAGase (left) and LDH (right).

Milk yield

Milk yield was measured in 648 udder quarters. The median milk yield was 0.38 kg (IQR: 0.25 – 0.51 kg) and the mean was 0.39 ±0.20 kg. The highest milk yield measured in one udder quarter was 0.98kg. There was no significant association between milk yield and CMT (Figure 5) or between milk yield and SCC.

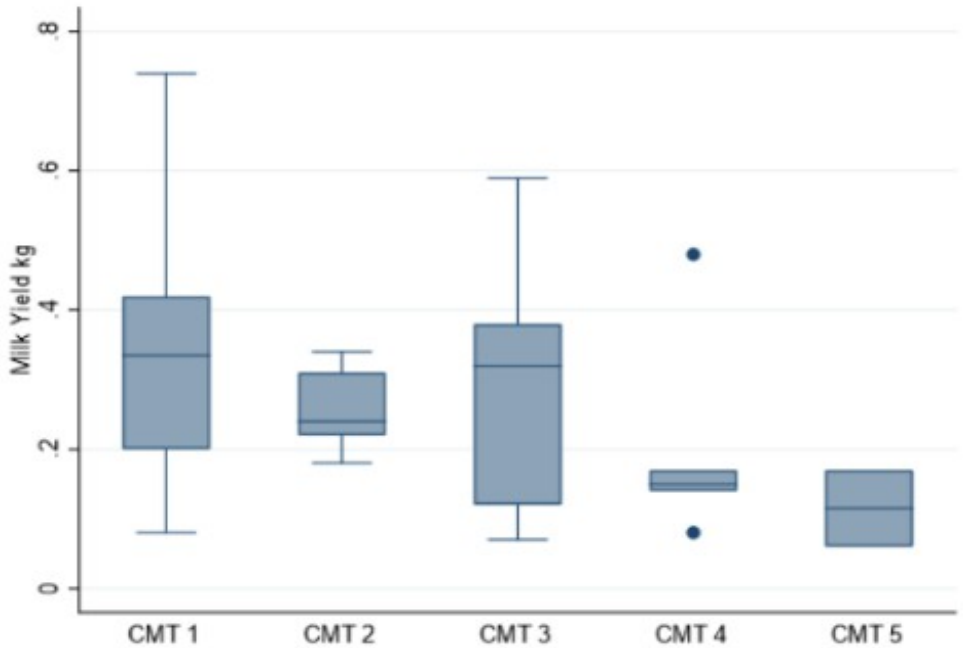

Figure 5: Box-and-whisker plot of milk yield in milk samples with CMT score 1-5.

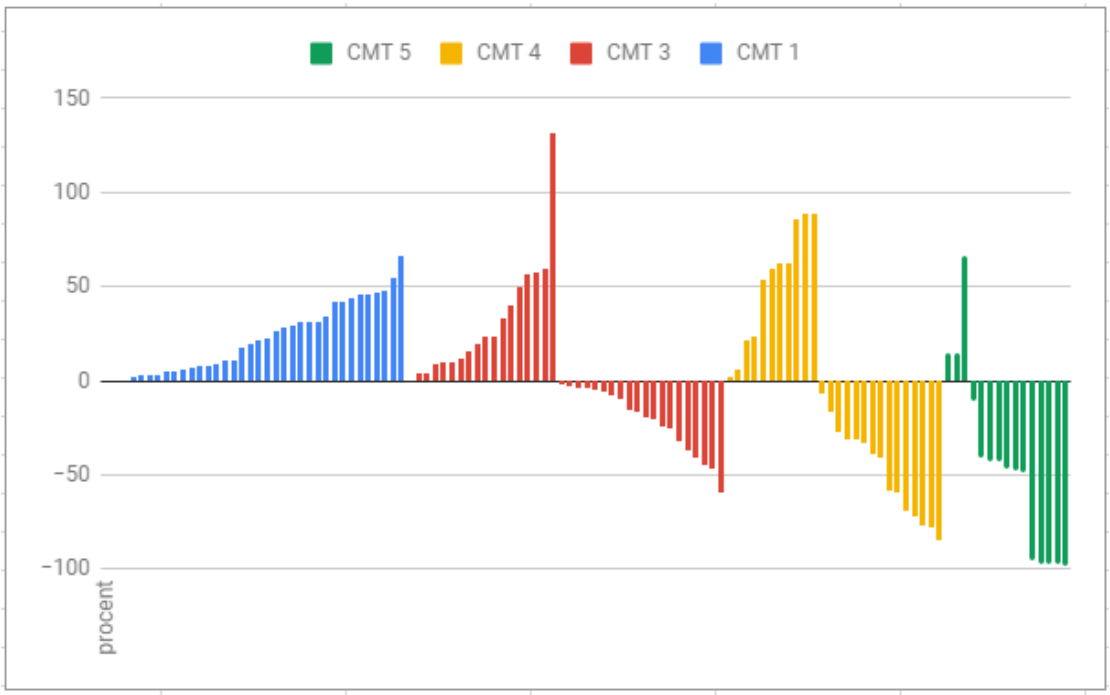

Figure 6: The difference in milk yields between front- front or hind-hind teats is shown in Figure 6, where 0% represents no difference in milk yield between the compared teats. The blue CMT 1 category is the percentage difference in yield between two healthy quarters. The negative value for quarters with CMT 2, 3, 4 and 5 indicates the reduction in milk yield from the subclinical mastitis quarter t compared to the healthy quarter (CMT 1), whereas a positive value for quarters with CMT 2, 3, 4 and 5 indicates that the subclinical mastitis quarter is producing more than the healthy quarter.

With a CMT 5 in one of its paired udder quarters, the milk yield was, on average, 44.7% (maximum 66% and minimum 0%) less in the quarter with subclinical mastitis (n=15). With a CMT 4 in one of its paired udder quarters, there was, on average, 6.6% (maximum 131% and minimum -59%) less milk in the quarter with subclinical mastitis (n=26). For a CMT 3 in one of the paired udder quarters, there was, on average, 3.5% (maximum 89% and minimum -85%) more milk in the quarter with subclinical mastitis (n=38). The average difference in milk yield between two healthy quarters (CMT 1) was 22.4% (maximum 66% and minimum -98%) (n=36).

Due to time limitation, the CMT measurements were not performed with each milk yield measurement but were conducted at the first herd visit in all herds. In herd A the next CMT were conducted after 7 days and all the udder quarters that still showed CMT 1 were then counted as CMT 1 on the two subsequent days. In herd B the next CMT measurements were conducted after 12 days and in herd C after 10 days. Herd D did not have a second CMT measurement, due to time limitation. The two CMT results showed that, in most cases, if a camel had CMT 3 or more at first measurement, the CMT did not decrease during the 10-12 day period.

Temperature

The environmental temperature varied from 17°C to 23°C and was not taken into account when handling the udder skin temperature data. There was no clear pattern regarding the temperature and the milk yield (Figure 7)

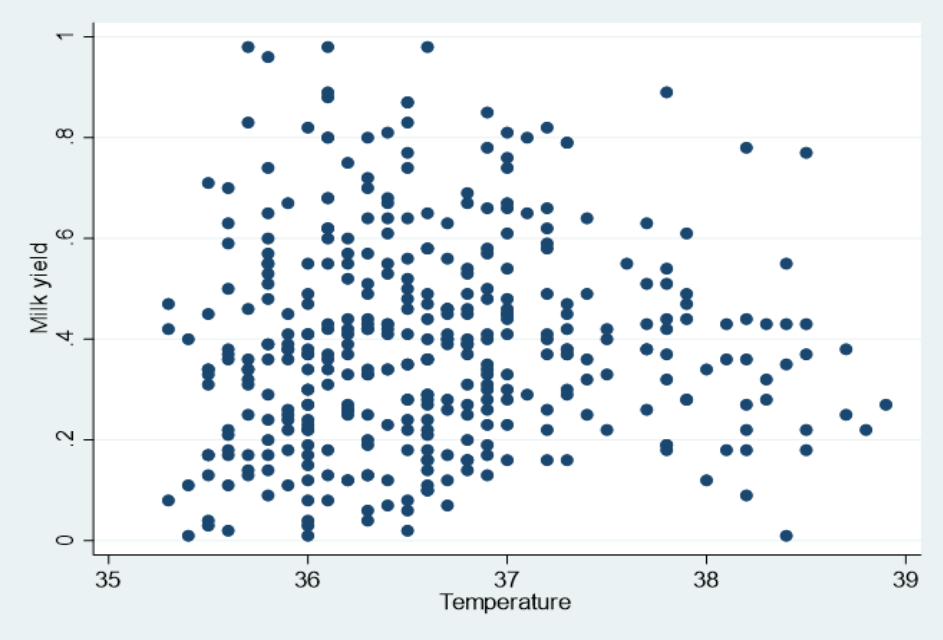

Figure 7: Box-and-whisker plot of the association between udder skin temperature in udder quarters and milk yield.

Questionnaire

The mean number of calves that the camels had produced, the mean lactation month in which the sampling occurred, and the means of the herdsman´s evaluation of the camel´s production (Low =1, Medium =2 or Good =3), compared to the camel´s highest CMT score of the four quarters are shown in Figure 8.

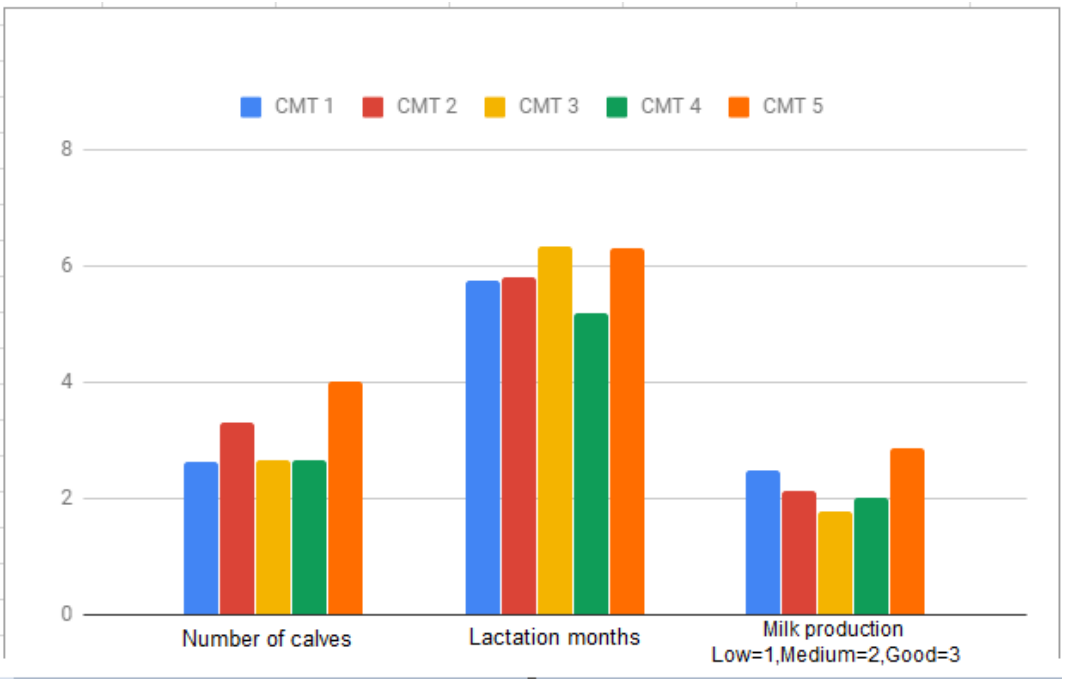

Figure 8: The average number of calves, average lactation month and average milk production for camels with different CMT scores.

Figure 9 shows the distribution of CMT related to the number of calves, the lactation month and the herdsman´s own evaluation of the camel´s production, low, medium or good (values indicated on the y-axis). Compared to Figure 8 showing the mean score of the answers, Figure 9 shows the herdsmen´s responses and the number of camels that were included in each CMT group. The number of camels in each CMT group shows how common that CMT score was for that question.

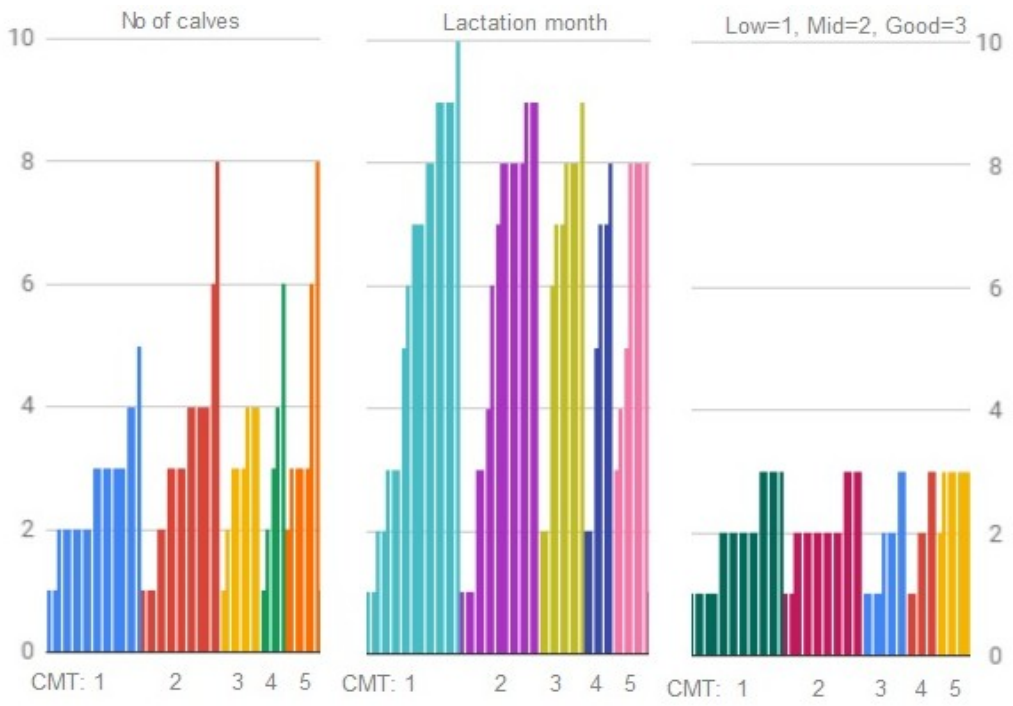

Figure 9: Relationships between the number of calves, the lactation month, and the herdsman´s own evaluation of the camel´s production. Each category shows the number of camels that was included in the CMT group.

The herds that were included in the study was seen to have inconsistent milking and hygiene routines. There was a lack of milking order, milking time, sanitizing of equipment, or the possibility to cool the milk. In addition, four of five herds used disposable plastic containers to store and transport milk, instead of metal containers. There was a lack of knowledge of methods for controlling the occurrence of subclinical mastitis. Also some tribes have a tradition of not selling or slaughtering the female camels, resulting in retention of camels that were affected with mastitis or with blind teats.

Discussion

Subclinical mastitis is a major challenge to camel dairy production for pastoralists in Kenya. The aim of this study was to investigate if CMT could be of use to identify udder quarters with subclinical mastitis and if there were any associations between subclinical mastitis and quarter milk yields in dromedaries in pastoralist herds in Kenya. There was a significant relationship between CMT with SCC, NAGase and LDH, showing that CMT would be a sufficiently accurate tool to be used in the field to detect camels with subclinical mastitis. The SCC had the strongest connection with CMT score, compared to NAGase and LDH. This is logical because CMT is a way to measure SCC. Subclinical mastitis was measured by CMT scoring as well as with the inflammatory markers SCC, NAGase and LDH. However, CMT 1, 4 and 5 had the most distinct linear correlation with these markers, whereas CMT 2 was harder to distinguish by observation, perhaps because it represent a transition zone. Udder skin temperature did not have any relationship with milk yield.

The SCC for *Camelus dromedarius* investigated in the present study correspond to the SCC for cows, according to both international CMT and Scandinavian standards, as shown in the literature review (Goncalves, 2017). However, the interquartile range showed a slightly lower median interval for camel (<112,000 cells) than for the SCC interval for cows (<200,000). Hamed *et al.* (2010) did see that camel milk had a lower mean value of SCC than dairy cows when both animals were studied in two groups with high and low SCC. It appears that CMT is a good marker for subclinical mastitis.

The SCC was lowest in the right front quarter and highest in the right hind quarter. The part of the udder that had the highest prevalence of mastitis was significant. The percentage difference in milk yield between front and hind quarters (40%-60%) did not explain the SCC difference between front and hind quarters.

Both the enzymes NAGase and LDH are significantly associated with SCC, in agreement with Hovinen *et al.* (2016), who stated that NAGase could be used with an accuracy of 85-99% for subclinical and clinical mastitis. Also Nyman *et al.* (2014) showed that NAGase and LDH are higher in mastitic cows than in cows without any intramammary infections. Earlier observations of the value of NAGase and LDH in detecting mastitis were made by Bogin *et al.* (1973) and Kitchen *et al.* (1978). The median(IQR) values of NAGase and LDH in healthy udders, in the present study were 16.5U/L (13.6-21.2), and 8.8U/L (7.2-12.6). Mean values for NAGase and LDH in goats for early, mid and late lactation were 1.9, 1.3 and 4.5 U/L, and 3.2, 3.3 and 7.7 U/L, respectively (Persson *et al.,* 2014). Mean (SD) values for cows were 4.69 (2.21) U/L and 1.73 (1.34) U/L (Nyman *et al.,* 2014). The enzyme levels for camels are higher than for goats and cows, but it was not possible to determine if this depends on the type of animal or the study environment. Both NAGase and LDH were significantly associated with CMT scoring, indicating that CMT would be a good marker for subclinical mastitis in the dromedary camel. However, there were too few NAGase and LDH measurements to see any association with milk yield.

The results of the milk yield from the camels were in agreement with earlier studies by Onjoro *et al.* (2006) and KARI. There was no significant association between milk yield and CMT scores or between yield and SCC. The percentage difference in milk yield between CMT 4 or CMT 5 quarter and a CMT negative quarter indicated that a high CMT is associated with a lower milk yield. That CMT 3 quarters produced more milk than their paired healthy quarters is surprising. However, the average differences in milk yield between

two healthy quarters (CMT 1) was 22.4%, indicating that, in general, there is a big difference in milk yield between quarters. Forster *et al.* (2010) used the same method for dairy cows to estimate percentage milk lost due to CMT 3 or more, in paired quarters. The results in Forster ´s study showed a decrease in milk of 9.0% for CMT 2, 19.5% for CMT 3, 31.8% for CMT 4 and 43.4% for CMT 5. The percentage loss for CMT 5 in the present study is in agreement with the results of Forster *et al.* (2010). An explanation of the increase in milk yield for CMT 3 could be the large variation in milk yield between the paired quarters. To our knowledge, studies on milk yield differences between paired healthy quarters in camels have not been done before.

By knowing that CMT 4 and 5 exist in 8% of all 505 udder quarters that were tested in this study and that milk yield decreases in average 6.6% (CMT 4) and 44.7% (CMT 5), the impact on the pastoralists´ economy can be considered. The average milk yield is 0.39 kg ~ 4 dl. The herdsmen are paid approximately 100 Ksh per liter of raw milk, or 40 Ksh for 4 dl, which is approximately 4 SEK. A camel´s lactation period is 11.8 months, on average, but could last up to 18 months. Thus, on average a herdsman can lose from 960 Ksh (96 SEK) to 6500 Ksh (650 SEK) per udder quarter that contain subclinical mastitis every year. A camel can produce up to 12 calves which means 12 lactation periods. Also a lost quarter due to mastitis could mean 14,600 Ksh (1,460 SEK) lost income every year. These numbers are based on the milk yield for camels in this study in the dry season, with one milking per day. A greater loss in income would be found if the calculation is based on the milk yield during the wet season, which could be double that in the dry season. The minimum wage allowed in Kenya according to *Tradingeconomics* is 13,572 Ksh (1,357 SEK).

One observation that could explain the differences in milk yields within camels could be the milking performance of the herdsmen. The milk bucket that was constructed for this study worked well. After a test-run, replacing the funnels with ones which had a more conical shape allowed the milk to be collected without any waste. The herdsmen understood the system well, and were able to milk one teat into each funnel. The milking was also monitored by the responsible supervisor. The herdsmen were milking two by two, with one herdsman on each side of the camel. One herdsman helped to stimulate the camel and was ready with the bucket, while the second herdsman was focused on the calf and the camel herd. The calf was allowed only to suck enough to stimulate milk let–down without getting any milk; the second herdsman watched it carefully and, while milking, he also had to push the calf away from the udder continually. Due to this problem, milking was not done in an equivalent manner on both sides of the camel. Another factor involved in uneven milking of the camel´s right and left side, is the herdsmen´s habit of chewing “Mirra”. “Mirra” is a leaf that contains a stimulant drug that could affect the engagement of the chewer on his task.

The udder skin temperature measurements in the study did not show any clear pattern regarding an association with milk yield. In previous studies with cows (Sathiybarath *et al.,* 2016; Polat *et al.,* 2010; Hovinen *et al.,* 2008), goats (Caruolo et al., 1990) and sheep (Castro-Costa *et al.,* 2013; Mala *et al.,* 2009), the infrared thermometer was shown to be useful as an early indicator of mastitis. Samara *et al.* (2013) found that camels with subclinical mastitis had an udder surface temperature 1.42°C higher than a healthy camel. The result from the present study did not show that the infrared thermometer ”Microlife NC150 non-contact thermometer” was a good indicator of subclinical mastitis. Due to time limitations, the CMT could not be measured the same day as udder temperature, and milk yield did not show any association with temperature. This could be due to the low number of animals in the study or

the time of the measurements. If a long-term study were to be performed where the udder skin temperatures could be monitored and controlled within the same camel, it is possible that a different result could be obtained. Further investigations are needed.

The questionnaire provided additional knowledge to the CMT, such that the camels with a higher parity number also had a numerical higher CMT score. This could be due to both a physiological variation well known in cows and/or a difference in the prevalence of mastitis. Ahmad *et al.* (2012) showed that parity number had a significant relationship to mastitis in camels, while Obied *et al.* (1996) did not find any physiological difference in SCC with increasing lactation number in mastitis-free camels

The result of the CMT scoring during the lactation period in the present study, gives unclear information; a larger number of camels would be preferable. Ahmad *et al.* (2012) found a significant relationship between mastitis and lactation stage in camels while Obied *et al.* (1996) did not observe any relationship between SCC and stage of lactation in healthy camels. From the herdsman´s own evaluation about the camel´s level of production in the present study, the camels that were said to be high milkers also had a high CMT score. However, the udder quarter samples showed a relationship between low SCCs and a high milk production, indicating that the perception of the herders might be wrong, or possibly that the camels with CMT 5 in one udder quarter produce more milk at the whole udder level. High-producing dairy cows are often more prone to mastitis and perhaps it is the same for camels.

The main difference in milking practice observed between the herds was the use of hand sanitizer in herd A. The herdsmen were using the sanitizer between milking different camels, which likely prevented transmission of bacteria from one camel to the next, which could explain the better udder health in this herd. There are several obstacles to including additional hygienic routines in the milking process. First, an understanding of why hygiene is important to the dairy market and for the individual camel is needed, to be able to prevent subclinical mastitis as well as to treat clinical cases. Knowledge of how bacteria are transmitted between the animals and herds is necessary, and the people who handle the animals need to be educated about hygiene. The average highest and lowest temperatures in the Laikipia district in Kenya are between 25-26°C and 9-10°C, respectively (Weather statistics YR 2018). The temperature at which *S. aureus* 2064 grows is 8-46 °C (Medvedova *et al.,* 2009). This means that there is always a beneficial climate for growth of bacteria in milk. Due to the tradition of not selling or slaughtering female camels, those animals with very severe or chronic mastitis problems remain in the herd and could transmit bacteria to the rest of the animals for many years.

A suggestion in order to improve the hygiene of camel milk would be for the dairies to place a higher demand on low SCC in the delivered milk, as already done in many countries. A common way today in at least the smaller dairies in Kenya is that the dairy has a person who smells and tastes the milk to decide if it should be approved or not. Introduction of a payment-based system would encourage low SCC because of its higher value. The owners of the herds would be interested in gaining as much profit as possible, and therefore take action against high SCC. A CMT-kit with a paddle is a very cheap and easy way to check the subclinical mastitis situation in the camel herd. Establishing a milking order would be beneficial, with the healthiest animals being milked first and those with higher CMT last.

Herd A was the herd with lowest prevalence of subclinical mastitis and also the only herd

where the milkers used hand sanitizer frequently. No studies have been done to compare herds with and without hand sanitizers yet, but it could be an interesting continuation to the present study. If use of the hand sanitizer is related to a lower cell count, it would be a cheap and easy way of preventing bacteria spreading between the camels on the hands of the milkers. Another improvement that could be done is to use metal containers instead of disposable plastic containers, since metal would be more hygienic and easier to clean.

The environment around the camels is beneficial for control of subclinical mastitis. The camel´s udder is attached high up between its hind legs. During the study, it was observed that the camels´ udders did not touch the ground. The camels´ faeces were dry; the udder was not contaminated with either faeces or urine. The risk for the camel to accidentally step on the teats was unlikely, because of the height of the udder. All these environmental risk factors are ways for mastitis bacteria to enter the udder, but were observed to be low during the study.

Conclusion

The results presented in this study show reality in the arid and semi-arid parts of Laikipia district in Kenya, rather than in intensively farmed dairy camels. The inflammatory markers SCC, NAGase, LDH and CMT are good markers for subclinical mastitis for *Camelus dromedaries,* although udder skin temperature did not work well as a marker in this study. The milk yield did not show any significant relationship with CMT or with SCC. However, the percentage difference in milk yield between the paired udder quarters showed that a high CMT score could be associated with a decrease in milk yield.

As CMT is an easy and cheap way of detecting udder quarters with subclinical mastitis,that could be used in pastoral camel herds to improve udder health and camel milk quality in Kenya.

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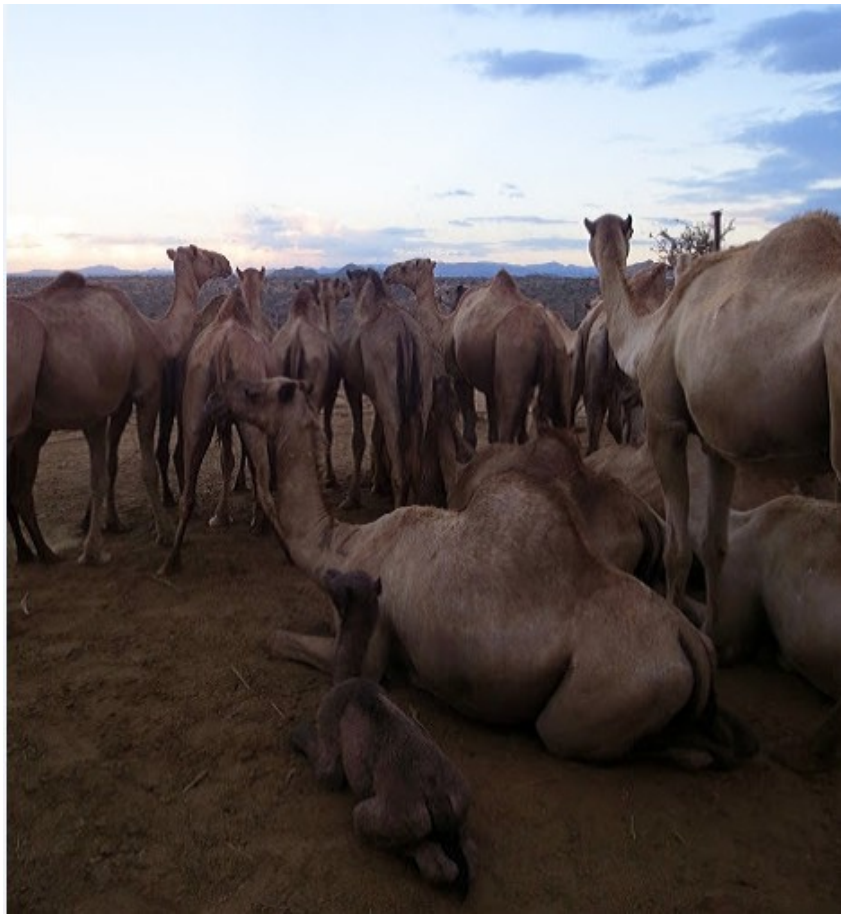
Udder health inflammatory markers in camel milk (*Camelus dromedarius*) and milk yield



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External mentor: Mario Younan

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The staff at Mpala Research Center
and The guard Davis
"-Asante Sana"

Supervisor Jane Morrell

Epidemiologist Ann Nyman

External mentors Mario Younan
and Set Bornstein

The project group at SVA

Abstract

Kenya is one of the biggest producers of camel milk in the world. Apart from milk production, camels are also a very important source of food and income for pastoralists. Camels (*Camelus dromedarius*) are well adapted to the harsh environments and arid parts of the country. Mastitis is one of the most common and costly diseases of dairy animals because of loss in milk yield and cost of treatment. The quality of the milk also decreases due to mastitis and the milk will be worth less. Mastitis can affect the storage life of the milk, which can lead to a loss in income. The aim of this literature review was to obtain a greater understanding of why camel milk has become so popular and what challenges the milk industry in Kenya must overcome. The aim of the field study was to investigate if there were any associations between the inflammatory markers, somatic cell count (SCC), N-acetyl-B-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH), udder skin temperature or the California mastitis test (CMT), and subclinical mastitis or decreased quarter milk yields in affected quarters in camels. Descriptive statistic of the distribution of the inflammatory markers and milk yield were performed as well as statistical analyses of associations between each inflammatory marker and milk yield. The inflammatory markers SCC, NAGase, LDH and CMT appeared to be good markers for subclinical mastitis in *Camelus dromedarius*. The udder skin temperature did not work well as a marker for subclinical mastitis in this study. Milk yield did not show any relationship with CMT or with SCC. The percentage difference in milk yield between paired udder quarters nevertheless indicated that a high CMT was associated with a decreased milk yield up to 44.7%. However, more research is needed. As CMT is an easy and cheap way of detecting udder quarters with subclinical mastitis, it could be used as a measurement to improve udder health and camel milk quality in pastoral camel herds in Kenya.

Sammanfattning

Kenya är en av de största producenterna av kamelmjölk i världen. Förutom mjölkproduktion är kameler också en mycket viktig källa till mat och inkomst för pastoralister. Kameler (*Camelus dromedarius)* är väl anpassade till de hårda miljöerna och de torra delarna av landet. Mastit är en av de vanligaste och mest kostsamma sjukdomarna inom mjölkproduktionen pga den förlorade mjölkmängden och kostnaden för behandlingar. Mjölkens kvalitet minskar också pga mastit och mjölken blir mindre värd. Mastit kan också påverka mjölkens lagringstid, vilket kan leda till inkomstförluster. Syftet med denna litteraturöversikt var att få större förståelse för varför kamelmjölk har blivit så populär och vilka utmaningar mjölkindustrin i Kenya måste övervinna. Syftet med fältstudien var att undersöka om det fanns några samband mellan de inflammatoriska markörerna, somatisk cell count (SCC), N-acetyl-B-D-glukosaminidase (NAGase) och laktatdehydrogenase (LDH), juverhudstemperaturen eller California mastit test (CMT), och om de hade en relation med subklinisk mastit eller minskad mjölkmängd på juverdels nivå hos kameler. Beskrivande statistik över fördelningen av de inflammatoriska markörerna och mjölkmängd utfördes såväl som statistiska analyser eller med föreningar mellan varje inflammatorisk markör och mjölkmängd. De inflammatoriska markörerna SCC, NAGas, LDH och CMT är bra markörer för subklinisk mastit för Camelus dromedarius. Hudtemperaturen fungerade inte som en markör för subklinisk mastit i denna studie. Mjölkmängden visade inte något samband med CMT eller SCC. Den procentuella skillnaden i mjölkmängd mellan parvis jämförda juverdelar visade dock att ett högt CMT var förknippat med en reducerad mjölkmängd på upp till 44,7%, men mer forskning behövs. Eftersom CMT är ett enkelt och billigt sätt att hitta juverdelar med subklinisk mastit, kan det användas av pastoralister i kamelbesättningar, som en mätning för att förbättra hälsan och kvaliten på kamelmjöl i Kenya.

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Introduction

Dromedary camels (*Camelus dromedarius*) are well adapted to hot climates and arid environments due to their unique physiological, anatomical and behavioural characteristics. Camelids originate from North America. There are two groups of camelids: one which migrated to the west through the land connection between America and Asia, today known as the Bering straits, and the other group which migrated south and eventually developed into the South American camelids, SACs, or New World Camels (NWC). They all have the ability to ruminate (Odöö and Bornstein, 1993) but unlike the true ruminants such as cows, sheep or goats that have four "stomachs", the camelids have only three "stomachs" (Ross *et al.,* 1979). The western camels developed into the one-humped camels (*Camelus dromedarius*) and the two-humped camels (*Camelus bactrianus*) and (*Camelus ferus*), whereas the camels that migrated south, which do not have a hump, developed into the llamas, alpaca, vicuna and guanaco,. Llamas and alpacas are domesticated animals, used as pack animals and for meat and wool production, while vicuna and guanaco are mostly found in the wild (Odöö and Bornstein, 1993). The Dromedary camels are able to cope with hot, dry weather, and are mostly found in the northern and eastern parts of Africa, the Middle East and Central Asia. The Bactrian camels are better able to cope with cold weather and are mainly concentrated in China and Mongolia. The Old World Camels (OWC) provide several products as milk, meat, wool, bone and dung, as well as being working animals on farms, for carrying goods, riding and also for tourism (Odöö and Bornstein, 1993).

The camel population is estimated to be about 25 million; of these 95% are dromedaries (Faye, 2013). They provide milk which is a very important product for the nomadic pastoralist economy (Musinga *et al.,* 2008). The dromedary camel breed Rendille can produce more milk than four Zebule cows during the dry season and camel milk has a higher economic value than cow milk (Spencer, 1973). Camels are mainly browsers and with their height they are able to reach feed that other livestock cannot; thus they do not compete for forage with other ruminant livestock (Odöö and Bornstein, 1993). With a decreasing food production in Africa per capita and an increasing human population, keeping dairy camels is a sustainable way to develop food production in semi-arid and arid lands (Schwartz *et al*., 1992)

This study is a student project for a master thesis. The thesis was developed from an on-going PhD project with the title: "Control of *Streptococcus agalactiae* to reduce subclinical mastitis in pastoralist camel herds in Kenya". The sampling and practical work for this master thesis were performed in Laikipia district in Kenya. The aim of the study was to investigate if there were any associations between the inflammatory markers, somatic cell count (SCC), N-acetyl-B-D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH), udder skin temperature or the California mastitis test (CMT), with subclinical mastitis or decreased quarter milk yields in affected quarters in camels. The methods that were used were both existing, well-tried methods, and new but not yet so well-recognized methods described in studies of udder inflammation in camels. The aim of the literature study was to review these inflammatory markers and, in addition, explain why camel milk has become so popular and what challenges the milk industry in Kenya has to overcome.

Literature review

Background

Kenya is one of the biggest producers of camel milk in the world (FAO 2018). Camels are well adapted to harsh environments and to the arid parts of the country. They have also become a very important food and income source for the pastoralists with the increasing commercialization of camel milk (FAO 2018). The camel industry in the Isiolo district in Kenya has grown considerably in the last three to four decades as pastoralists are increasingly using their camels for commercial milk production. This development has arisen because camels are very mobile animals, which is necessary in areas with dry climate and poor feed (Musinga *et al.,* 2008).

More than 70% of Kenya consist of arid and semi-arid land, which is poorly suited to agricultural farming (Ominde *et al.,* 1988). About 10% of Kenya´s population are pastoralist (Kirkbride & Grahn, 2008). In the past, pastoralists mainly herded cattle and small livestock, but due to changes in the climate with long and hard droughts, the one-humped camel (*Camelus dromedarius*) has become more common as a climate-resistant dairy animal (Faye, 2012). During the severe drought in Kenya in 1984, pastoralists who were concentrating on camel production lost fewer animals than the households that kept cattle, goats and sheep (Fratkin et al., 1990). Another severe drought in Kenya led to huge losses in the livestock industry. Death rates from 40 to 70% for sheep, goats and cattle were recorded after the drought (Serena, 2011).

The camel's ability to cope with a very dry climate is due to its capacity to save body water, which stems from both behavioural and anatomical adaptations (Wilson, 1998). Camels prefer to browse during the night when the temperature is lower. However, this practice is not common among herded camels for safety and practical reasons (Wilson, 1998). The camel's body fat is concentrated in the hump instead of covering the whole body, making it easier to reduce body heat loss from the skin surface. The camel can also change its body temperature up to 6°C depending on the outside temperature, resulting in reduced water losses from the surface of the skin (Wilson, 1998). In addition, the camel can recycle water, by absorbing it from both faeces and urine. The camel does lose its appetite and milk yield when dehydrated, but these occur after a week of dehydration (Bekele *et al.,* 2011), in contrast to cattle which lose their appetite and milk yield after one day of dehydration (Steiger Burgos *et al.,* 2001). Camels can produce much more milk than cattle during dry conditions (Spencer, 1973).

The increased interest in, and demand for, camel milk in the last few decades have prompted a need to transport the milk over long distances. However, longer transport requires better milk quality (Musinga *et al.,* 2008). A common condition of the udder that affects the quality of milk is mastitis. Mastitis is defined as an inflammation of the udder, most often caused by infection by microorganisms, for example, due to an unhygienic environment or due to physical injuries in the udder (Sandholm *et al.,* 1995). Mastitis is one of the costliest diseases in the dairy cow industry because of the loss in milk yield, the cost of treatment with antibiotics and the cost of culling due to chronic inflammation (Jingar, 2017). In a study by Sinha *et al.* (2014), financial losses due to unsold bovine milk were caused by decreased yield and the decreased quality of the milk. In a study by Ma *et al.* (2000) it was shown that mastitis infections also affect the storage life of the milk, which can lead to a loss of income. Somatic cell count (SCC) is used to measure the level of inflammation and is a common health indicator for dairy animals (Sandholm *et al.,* 1995). When dairy cow milk with a SCC of 45,000 cells/mL was compared with milk that contained 849,000 cells/mL milk, a significant negative association was seen between level of inflammation and storage time,

despite the fact that both sets of samples had been pasteurised and homogenised. Moreover the samples with the highest SCC had a rancid and bitter taste after 21 days´ storage (Ma *et al.,* 2000).

A study on the camel milk industry in Isiolo District by SNV Netherlands Development Organization (Musinga *et al.,* 2008) concluded that the camel milk industry can permanently change the way of life of the inhabitants of the arid and semi-arid parts of the country. The demand for camel milk is growing, both nationally and internationally. However, various players in the milk chain, as well as milk organizations, need to develop to be able to market more milk (Musinga *et al.,* 2008)

Camel milk industry in Kenya

The camel industry in Kenya has grown considerably in the last three to four decades as camels have become more frequently used for commercial milk production by pastoralists (Musinga *et al.,* 2008). Several factors make camel milk popular: for example, people who are sensitive to lactose or are lactose-intolerant can drink camel milk without any adverse side effects. The milk contains a low level of the protein β-casein compared to cow´s milk and lacks β-lactoglobulin. These proteins cause an allergic reaction in lactose-intolerant people (Konuspayeva *et al.,* 2009).

Camel milk is also a popular healing drink. Pastoralists have always drunk camel milk, both as daily food and medicine (Guliye *et al.,* 2007). There is now an increasing interest from other groups of people. The suggested curative effects of camel milk are presented to a greater public by marketing it in popular magazines and articles online. “Headlines” describing all the benefits are listed; for example, it is claimed that camel milk avoids allergies in children, helps to fight autoimmune disorders and autism, has anti-aging and anti-diabetic properties, can cure tuberculosis and is good for weight loss (Wells, 2018; Ameya, 2017; Dubey *et al.,* 2016; Hall, 2011)

The population in Kenya has increased from around 15 million people in the 1980s to 51.5 million people in 2018 (Worldometer 2018). The high growth of the population and a pronounced migration into urban areas from the countryside has led to a growing demand for both food and camel milk (Noor *et al.,* 2012). The neighboring country, Somalia, started a decade-long civil war in 1991, which caused many refugees to seek safety in Kenya (Hammond, 2018). Somalians have a long tradition of keeping camels and drinking their milk (Guliye *et al.,* 2007), and Somalia has the world´s largest dromedary population (Faye, 2014).

A questionnaire about “Reproduction and breeding in dromedary camels” was completed by camel-keeping pastoralists in southeastern Nigeria in a field study by Abdussamad *et al*. (2011). The gestation period was 12-13 months for camels and the average age for camels at first calving was between 4 and 5 years. Moreover, the pastoralist stated that the average lactation period for camels was 11.8 months, although lactation length could vary between 8 and 18 months. The calving interval was, on average, 23.8 months. Females can breed for more than 20 years and have 8-12 calves during that time (Abdussamad *et al.*, 2011). According to FAO (2018), camels in Africa produce, on average, 1000 to 2700 liters of milk per lactation. The increasing use of camel milk and realization of its economic advantages in Africa were associated with an interest in reproduction and breeding (Atigui *et al.,* 2013).

In a study about the contribution of camels to the household diet of pastoralists, it was shown that half of the annual nutrition came from camels (Farah *et al.,* 2004). The household diet includes 84.5% of livestock products, with camel meat (24.6%) and milk (20.4%) making the biggest contribution, followed by 17.6% goat meat and 14.7% cow meat (Elhadi *at al.*, 2015). The average consumption of camel and cow milk varies depending on season. In the wet season, the consumption of camel and cow milk was 2.0 liters and 1.6 liters per day, respectively, while in the dry season the consumption of camel milk increases to 2.5 liters whereas cow milk decreased to 0.5 liters per day. Camel milk made the biggest contribution to the household diet in the dry season, at 28.2% of the intake. This is a consequence of a lack of supplies, for example, of vegetables, in the dry season (Elhadi *at al*., 2015).

The camel milk industry faces many challenges. Despite an expanding industry and a high demand, the hygienic aspects of food safety are not a primary concern for the majority of consumers (Musinga *et al.,* 2008). Raw camel milk is a traditional medical potion for many, and proper handling or boiling is not considered to be necessary. The potential to extend the camel milk industry, both for a broader range of customer and at a national level, requires a focus on hygiene (Musinga *et al.,* 2008).

Odongo *et al.* (2016) interviewed 235 people, including herdsmen and people from bulking and retailing centers, about camel milking routines in Isiolo town. The containers into which the milk is collected are made entirely of plastic: the reasons for this were stated to be because plastic containers are not as heavy as other materials (47%), or because plastic containers are inexpensive (36.3%). The lightness of the milking container is an important aspect for the milker as he holds it up with his knee due to the height of the camel´s udder. Also 17.3% of the dealers in the study by Odongo *et al.* (2016) claimed that plastic containers were good for preserving milk. After milking, the milk can be stored up to 11 hours before it reaches the first cooling station, often being carried by motorbike couriers in Isiolo town. The plastic milk containers are washed and held over the fire so that the number of microorganisms can be reduced by the smoke (Odongo *et al.,* 2016).

Odongo *et al.,* (2016) revealed many risk factors for microbial contamination along the milk chain. The study shows that the camel´s udder is often not wiped clean before milking and the hands of the milkers are normally not washed. The number of bacteria was lower on the camels´ teats than on the milkers´ hands. It was assumed that this was due to a cleansing action by the calf sucking to initiate milk let-down. However, it could also be that the calf introduces microbial contamination to the udder prior to milking (Noor *et al.,* 2013).

Another risk factor for bacterial contamination of camel milk is mixing of milk from healthy and mastitic udders. The overall opinion among participants in the report by Odongo *et al.* (2016) was that milk from sick camels is not a health risk and it would be a waste not to use it. The decision to use medication for a sick camel is often made by the herdsman himself without consulting a veterinarian. It is likely that milk from these camels contains antibiotic residues, which could lead to rejection at the milk collection center (Odongo *et al.,* 2016).

The camel industry in Kenya represents a market potential for pastoral women. The SNV (Netherlands Development Organisation) conducted a case study about pastoral women in the Kenya camel milk chain and the challenges for pastoral women. The men milk the camels and are responsible for production, whereas the women are responsible for the milk and are

active in the least profitable sections of the milk value chain, such as intermediary (80%), micro processing (30%) and local markets (30%) (Siloma, 2011).

One difficulty is the possibility for Kenyan women to obtain financial help from the bank. Many pastoral women are muslims and, according to the Islamic sharia rules, are not permitted to borrow money (Siloma, 2011). Another obstacle is payment for the milk. The transportation of milk for many women is done by public transportation, with the driver receiving the money for milk sold. The risks for the driver are high and robbery occasionally occurs (Siloma, 2011). Projects where money transfers are made to women by cell phone are being introduced to avoid robbery and to make the transactions more efficient. In addition, poor quality of camel milk is a challenge. Collaboration with herdsmen to improve milk hygiene is required (Siloma, 2011).

Another challenge in the camel milk industry is that the more commercialized production becomes, the closer to the dairy centers the camel herds need to be. Noor *et al.* (2012) used the expression "peri-urban production system" to describe how the pastoralist herding system is becoming more common closer to towns and cities. The closer the camel herds get to each other, the higher the density of the animals will be. Risks associated with a high density of camel herds close to each other include both that the vegetation will be over-exploited (Noor *et al.,* 2012) and that the transmission of disease will be higher between the herds (Bornstein, 2018).

Mastitis

Mastitis, inflammation of the udder, is often a result of a bacterial infection and can be classified as clinical, with visible symptoms, or subclinical, with non-visiblesymptoms. Clinical mastitis can be detected by visible signs, such as swellings of an udder quarter, changed milk consistency and colour, fever, etc. Subclinical mastitis is harder to detect as no visible changes can be seen. However, the milk composition is changed which can be confirmed by laboratory tests or cow/camel side tests (Guliye *et al.,* 2002). Clinical mastitis can be sub-classified depending on how severe the symptoms are (SVA 2018). In acute mastitis, the animal´s udder can be swollen and sore. The animal can be weak and slow, have a fever, and there are usually changes in the texture, color and odour of the milk. Milk yield can be reduced and the concentration of somatic cells in the milk will increase (Sandholm *et al.,* 1995). These symptoms are the same for several dairy animals, such as sheep (Gårdochdjur hälsa, 2018), goats (Svenska getavelsförbundet 2018a) and camels (Wilson, 1998). Untreated mastitis could develop into a chronic case. An acute clinical mastitis is often treated with antibiotics (SVA 2018). In severe cases of mastitis the cow may die or be culled (Jingar *et al.,* 2017). The prevalence of mastitis in milking camels in Kenya has been investigated in several studies (Toroitich *et al.,* 2017**,** Kaindi *et al.,* 2011; Abdurahman 1996). In Africa and in the Middle East the prevalence of clinical mastitis in camels varies from 24.1% (Almaw *et al.,* 2000) to 76.0% (Seifu *et al.,* 2010), and for subclinical mastitis in camels from 20.7% (Abera *et al.,* 2009) to 33% (Aljumaah *et al.,* 2011).

The camel has not previously been recognized as an animal for dairy research. As severe droughts are becoming more common and as demand on camel products increases, the need for research is growing. However, most of the research on subclinical and clinical mastitis is focused on dairy cows. These dairy animals have physiological and anatomical similarities that make research on dairy cows applicable to dairy camels as well (Bornstein, 2018). The

camel´s udder has four quarters, each having glands and gland cisterns as in the cow´s. The difference is that the camel has two glands per quarter each of which has its own separate "small" gland cisterns (Abshenas *et al.,* 2007), compared with the cow where there is only one gland and a "big" cistern per quarter (Sandholm *et al.,* 1995). Cow's milk has more lactose and protein than camel milk (Soliman, 2005). Camels have similar udder proportions to the cow (Bogucki 2017, Šlyžius *et al.,* 2013), with 60% of the milk yield originating from the hind udder quarters and 40% milk from the front udder quarters (Caja *et al.,* 2011, Eisa *et al.,* 2009). The biggest difference is the vitamin content, since camel milk has much more vitamin C than cow milk but lacks vitamin A. Camels are often compared with dairy cows when they are kept in the same environment (Faye, 2012).

The most common bacteria causing an elevated somatic cell count (SCC) in cows, sheep and goats in Sweden are *Staphylococcus aureus*, non-aureus *staphylococci* (NAS), and *Streptococci* strains, in approximately 80% of cases (Svenskagetavelsförbundet 2018b). The remaining 20% of cases are caused by environmental bacteria such as Klebsiella and *Escherichia coli* (Svenskagetavelsförbundet 2018b). Mastitis can be transmitted between lactating animals, depending on the type of bacteria (Sandholm *et al.,* 1995). One example is *S. aureus* that can be transmitted between dairy cows by the hands of the milkers (Gustavsson, 2012).

The main bacteria responsible for mastitis in camels in Kenya was investigated by Toroitich *et al.* (2017), who performed bacterial isolation from milk samples from 380 udder quarters; 114 bacteria isolates were found. The main finding was *S. aureus* with a frequency of 36.0%. The second most common finding was *E. coli* (27.2%). *Staphylococcus epidermidis* and *Streptococcus agalactiae* were found with a frequency of 9.6% each. Toroitich *et al.* (2017) claimed that the growth of mixed types of bacteria in the milk indicated a multiple infection in the sampled quarters.

An immunocompromised dairy animal is more sensitive to infections. Mastitis may occur when the lactating animal is in a sensitive stage, e.g. dairy cows are at a bigger risk for mastitis at the beginning of the dry period or the beginning of lactation (Sandholm *et al.,* 1995). Accordingly, Ahmad *et al.* (2012) found that stage of lactation and parity number had a significant relationship with mastitis also in camels and that the prevalence of mastitis was highest during the initial and last stage of lactation. Stress due to the animals´ situation could be a factor lowering resistance. Breed and lack of hygiene during milking were shown to be associated with increasing risk for mastitis in camels (Ahmad et al., 2012). In cows there is a physiological variation in SCC with stage of lactation and lactation number (Sandholm *et al.,* 1995). Obied et al. (1996) did not find any such physiological significant difference in SCC during the lactation or between lactation numbers (Table 1) in camels that were defined as free from mastitis by CMT and bacteriological examination.

Stage of lactation (months)	Lactation number 1	2	3	4	5	6	7
1-4	108 (28) [12]	120 (25·3) [18]	107 (22·1) [10]	213 (37·8) [12]	150 (21·1) [9]	212 (39·6) [11]	275 (54·9) [8]
5-8	50 (18) [28]	125 (33·1) [17]	207 (43·2) [41]	125 (26·4) [24]	200 (28·8) [21]	150 (38·4) [15]	350 (75·2) [16]
9-12	200 (48) [9]	144 (27·2) [10]	190 (34·5) [16]	144 (29·2) [8]	200 (36·6) [17]	220 (36·6) [8]	225 (48·1) [14]

Table 1: *Somatic cell counts (cell/ml) in camel milk during the lactation period and between successive lactations (Obied et al., 1996)*

An udder close to the ground could be a risk factor, due to close contact with environmental dirt and soil bacteria. Odongo *et al.* (2016) claimed that the camel´s udder is in contact with the ground while the camel is lying down. Porcionato *et al.* (2010) studied the relationship between teat morphology and the prevalence of mastitis in cows. Cows with longer teats had low-hanging udders and higher SCC. Injuries such as scratches and cuts on the udder could also start an infection that leads to mastitis (Beef and lamb 2018).

The risk factors in a dairy herd should be evaluated to prevent mastitis. Hygiene around milking and of milking equipment is essential. Healthy animals should be used as breeding stock, and provided with nutritious feed and water. If the bacteria causing mastitis in a herd are contagious, transmission can be avoided by milking animals that have low SCC before animals with a high SCC (Sandholm *et al.,* 1995). For cows, it is recommended to avoid giving female calves the milk from cows with mastitis caused by *S. aureus* (Barkema *et al.,* 2009). Keeping cows standing for half an hour after milking instead of allowing them to lie down will give the teat canal time to close before it is exposed to dirt or bacteria (Blowey *et al*., 1995). Chronic mastitis-affected dairy cows are contagious for the herd and should be culled (Sandholm *et al.,* 1995). These factors could also be applied to camel husbandry.

California mastitis test and somatic cell count

Somatic cell count increases in the udder quarter during an inflammation and is an indicator of subclinical mastitis. The SCC can be measured directly using cell counters or indirectly by the California Mastitis Test (CMT), which are ways to check the inflammatory status in lactating camels (Abdurahman, 1996). These methods are used routinely to detect udder inflammation in several dairy animals, such as cows (Jánosi *et al.,* 2004), sheep (Pradieé *et al.,* 2012), goats (Persson, 2015), and buffalo (Dhaka, 2006). The CMT test can be used easily in the field for a quick and cheap result for the pastoralists. In contrast, expensive analytical instruments are needed to be able to measure the actual cell number.

When an inflammation in the udder is triggered, commonly by an infection with microorganisms, the leukocytes (white blood cells) will increase as a defence mechanism.

This increased number of leukocytes in the milk can be measured. The CMT test is a cow-side test based on a reagent added to the bovine milk which will disrupt the cell wall, allowing the DNA and to leak out and change the viscosity in the mixture. The more cell contents that are released, the more the mixture thickens. The CMT test is highly correlated with the level of SCC in milk (Plummer *et al.,* 2012).

The increase in SCC in camel milk was shown to be similar to the increase in cattle milk during an udder inflammation. Therefore, CMT can be used to check the inflammatory status in camels (Abdurahman *et al.,* 1995). The CMT was first described by Schalm and Noorlander (1957). The scale is divided into 5 score levels, all of which are denoted by numbers (1-5) according to the Scandinavian CMT recommendations (Table 2, Goncalves, 2017). The distribution of CMT values for dromedary camels in Kenya in a study by Goncalves was as follows: 1:52%, 2:37%, 3:8% 4:3% and 5:0% of 253 camel quarters (Goncalves, 2017). The frequencies of CMT values reported by Woubit *et al*. were 1:71%, 2: 23%, 3:4%, 4:0.1% and 5:0% (Woubit *et al.,* 2001).

Scandinavian CMT – score	International Score	Criteria	SCC (cells/ml)
1	Negative	No thickening or gel formation, fluid stays homogenous	0 – 200,000
2	Trace	Mild thickening of fluid when vessel is tilted	200,000 – 400,000
3	1	Clear thickening of fluid when vessel is tilted	400,000 – 1200,000
4	2	Clear thickening of fluid with a tendency of gel formation that disappears when vessel is not rotated	1200,000 – 5000,000
5	3	Clear thickening and gel formation that remains when vessel is not rotated	>5000,000

Table 2: *International CMT scoring and the Scandinavian CMT scoring systems and their criteria. The SCC (cells/mL milk) compared to the CMT scoring are value from dairy cattle (*Goncalves, 2017).

In studies by Merle *et al.* (2007) and Bansal *et al.* (2005), cows with a SCC below 100,000 cells/ mL milk were considered to be healthy, whereas cows with SCC higher than 100,000 cells/ mL milk in one of the udder quarters were considered to have inflammation. Hamed *et al.* (2010) compared low and high SCC between cow and camel milk. Two groups were created, one with SCC ≤ 105 cells/mL and the other one with SCC ≥ 105 cells/mL. Camel milk had a lower mean value of SCC in both the high and low SCC groups. The SCC for camels in comparison with the cows in the lower scoring group was 25.5±16.4 $x10^3$ and 32.5±23.9$x10^3$, respectively. In the high scoring group, the SCC for camels and cows were 331.4±436.7$x10^3$ and 369.1±433.2$x10^3$, respectively.

Many countries use SCC as an indicator of milk quality, with the farmer receiving either a reduction or an increase in payment for the milk based on the results. Countries that do not have milk payment based on milk quality often have poorer milk hygiene (Pasic *et al.,* 2016).

The biggest dairy in Sweden, Arla, has a 2% increase in commodity value if the SCC in the bulk milk is lower than 200,000 cells/mL milk. However, a level above 300,000 cells/ mL milk will result in a reduction in payment, which can be up to 10% if the SCC shows more than 400,000 cells/mL (Arla.se 2019).

A wide range of SCC in camel milk has been reported. In a study done by Merin *et al.* (2004) the mean SCC of milk from healthy camel udders was 118,000 cells/mL, and an udder with inflammation had a mean SCC of 308,000 cells/mL milk. Abduraham (1995b) reported that an average SCC in camel milk for quarter with no growth of bacteria was between 216,000 cells/mL and 415,000 cells/mL; camel udders with quarter milk SCC above 550,000 cells/mL should be considered to be infected.

In the early 1900s SCC was counted manually using the direct microscopic somatic cell count (DMSCC). This method is commonly used as a reference and was described first by Prescott & Breed. (1910). A small amount of milk is spread over a surface where it will dry, allowing the cells to be stained for observation and counting using a microscope. This method is still being used nowadays with some modifications (Gonçalves *et al.,* 2018, Abdurahman *et al.,* 1996). However, this technique is time-consuming and results can vary between different observers depending on how they interpret what they see in the microscope (Gonçalves *et al.,* 2018).

New techniques have been developed to make cell counting easier and faster, and to reduce differences in interpretation. The new Fossomatic 7 (FOSS) can count up to 600 milk samples in one hour. The FOSS technique uses flow cytometry where the milk cells are run through a capillary pipe and counted by photo electronics. The precursor to Fossomatic 7 was first developed in 1980s. The Fossomatic technique is used in milk testing centers (Fossanalytics, 2018).

DeLaval has developed an automatic optical cell counter (direct cell counter [DCC]), that is also portable. A picture is taken with a digital camera of the nuclei of the somatic cells which have been stained with fluorescent reagent and these are then counted individually. The count is displayed after 45 seconds (DeLaval 2003).

When DCC was compared with DMSCC on a large-scale camel dairy farm, there was a strong correlation between the cell counting measurements. The mean DCC (363,000 cells/mL milk) was slightly lower than for DMSCC 398,000 cells/mL milk. The two different methods had the same coefficient of variation of 23.5*%. (*Nagy *et al.,* 2013*).*

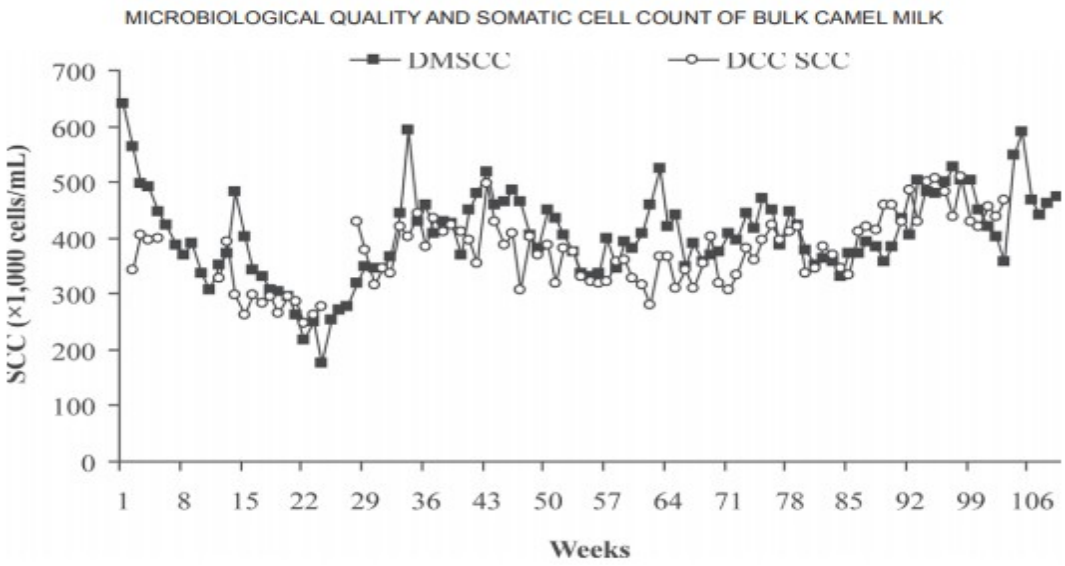


Figure 1: *The change in mean SCC per week for 2 years using the SCC methods DMSCC and DCC (Nagy et al., 2013).*

Relationship between mastitis and milk yield

The milk yield of camels was investigated in several studies (Zelek. 2007: Onjoro *et al.,* 2006; Bekele *et al.,* 2002) and shown to vary naturally during the lactation period. Other factors affecting the milk yield include the accessibility of feed and water, dry or wet season, age, and SCC (Zelek, 2007).

The camel's udder differs from that of other dairy animals, mainly in the ability to store milk. The camel stores about 90% of the total milk yield in the alveoli (Ayadi *et al.,* 2013*),* while many other dairy animals store a large amount in the milk cistern. Goats can store up to 75% in the cistern, cows 30%, and dairy sheep 50% (Costa *et al.,* 2003). When more milk is kept in the alveoli, a strong milk ejection reflex is necessary for complete emptying of the udder (Atigui *et al.,* 2016). Cows, goats and sheep have one teat canal attached to the udder cistern. The camel has two, sometimes even three, teat canals, each attached to a separate glandular complex, whereas horses and pigs have 2-3 teat canals each leading from a separate udder cistern (Husvéth, 2011)

In a report by Onjoro *et al*. (2006), the mean daily milk yield was 3.4±0.2 L/d (n=12) for free-ranging camels in northern Kenya at Kenya Agricultural Research Institute (KARI) field station. The milk yield increased from 3.4 L/d in the dry season to 4.3±0.3 L/d in the wet season. The recordings that KARI made on its own camels corresponded to a mean value at 3.2 L/d with Onjoro *et al.* (2006). The mean milk yield recorded for Somali camels in Ethiopia was reported to be 4.14±0.04 kg/day (n=61) (Bekele *et al.,* 2002), which is in agreement with the KARI and Onjoro *et al*. (2006) studies on milk yields for camels. Bekele *et al.* (2002) also reported that the daily milk yield in camels can be enhanced by increasing the number of milkings. With one milking per day, a production of 1.26±0.05 kg was recorded, whereas with four milkings per day the daily yield increased to 6.77±0.15 kg.

In a literature review by Hortet *et al.* (1998), data collected from 20 studies on milk yield losses and composition changes due to clinical mastitis in dairy cows were analyzed. Eight of the studies compared milk yield from cows with mastitis against the cows´ own milk yield

from a previous lactation. They also compared it with cows in the same herd that were considered healthy and had never been treated for mastitis. From these eight studies, five looked at the milk yield loss without considering the lactation number. The average losses due to mastitis varied from 0% to 9.5%. In nine other studies that took into account the cows´ lactation number, milk yield losses varied between 0.5% and 6.4%. Lucey *et al.* (1984) reported a milk yield loss of 11% when comparing the milk yield before the peak of the lactation curve with the whole lactation, as well as a 6.4% milk yield loss for cows with mastitis compared to those without mastitis. This type of study has not been performed on camels.

One reason why the milk yield decreases during mastitis is that when an udder inflammation occurs, the udder epithelial cells will be damaged. That means that the synthesis of lactose, fat and protein will be reduced and the milk will contain less of these substances (Sharif *et al.,* 2007) The reduction of lactose, which is the major osmosis-regulating substance in milk, results in a reduced amount of milk (Deluyker, 1991).

Forster *et al.* (2010) studied milk yield and CMT in individual quarters of dairy cows and compared a mastitic quarter with the opposite quarter with a negative CMT score. The study used measurements of one milking from 763 cows. The results were distributed between both front and rear udder quarters and throughout the whole lactation period. Udder quarters with CMT scoring of "trace, 1, 2 and 3" were shown to be related to a decrease in milk yield as follows: CMT trace, 9.0%; CMT 1, 19.5%; CMT 2, 31.8%; and CMT 3, 43.4%. Merle *et al*. (2007) and Bansal *et al.* (2005) reported that if a cow had one udder quarter with an infection, the other healthy udder quarter would also have a higher level of somatic cells than a quarter from an udder that is completely free from infection. In contrast, Barkema *et al.* (1997) stated that an intramammary infection (IMI) is often restricted to one udder quarter and does not spread to the other quarters by itself. However, they considered that transmission of contagious bacteria such as *Str. agalactiae* and *S. aureus* by milking equipment or between cows to be possible.

A severe case of mastitis or an unhealed teat injury could result in cessation of milk production from this quarter (Jones, 2009). Compensatory changes in the milk yield between cow udder quarters that were milked or not milked were investigated by Hamann *et al.* (1990). The average daily milk yield was measured in a pretreatment period for each cow included in the study; then one, two or three udder quarters were selected for non-milking. After the 12 days treatment period, another period of 12 days was initiated when the cows were milked from all four quarters again. The milk yield during the 12 days´ treatment period for the cows that were milked continuously from one, two and three quarters was increased by ~14%, ~10% and ~4% of the mean daily milk yield in the treatment period. The udders that were milked from one quarter produced 78% of their original average daily milk yield after the treatment period was over (Hamann *et al.,* 1990).

Enzymes NAGase and LDH

The enzyme NAGase is a lysosomal enzyme that is released if somatic cells, such as epithelial cells, are damaged and the cell content and plasma proteins leak out (Kitchen *et al.,* 1980, 1978). Another enzyme, LDH, is released during lysis of mammary cells (Singh *et al.,* 2015). During an infection in cows, the levels of NAGase and LDH will be much higher than in a healthy cow due to damaged cells (Chagunda *et al.,* 2006). The NAGase activity has also

been used as an inflammation indicator for ewes (Maisi *et al.,* 1987) and for goats (Timms *et al.,* 1985), and LDH- activity was described as a good indicator of subclinical mastitis in buffaloes (Singh *et al.,* 2016).

Hovinen *et al.* (2016) stated that both subclinical mastitis and clinical mastitis can be detected by NAGase with an accuracy of 85% and 99% in dairy cows. They also showed that the level of NAGase increases when the SCC increased. In the first 30 days of the cow´s lactation the NAGase activity was higher than in the rest of the lactation. Hovinen *et al.* (2016) also found that NAGase was higher in milk from older cows than from younger cows. This corresponded with findings in a report by Nyman *et al*. (2014) in which both the milk enzymes, NAGase and LDH, were higher in older cows than younger ones, and higher in mastitic cows than in those without IMI. The values for NAGase in IMI negative cows varied between 0.02-15.6 U/L whereas the IMI positive values varied between 1.28-15.3 U/L. The values for LDH in IMI negative cows varied between 0.15-12.0 U/L whereas the IMI positive values varied between 0.48-20.4 U/L (Nyman *et al.,* 2014). In a study by Åkerstedt *et al.* (2010), the NAGase and LDH in clinical healthy cows was 0.8-6.1 U/L and 1.1-3.2 U/L, resepectively, whereas the cows with subclinical mastitis had mean levels of 25.0 ± 28.9 U/L for NAGase and 45.0-58.9 U/L for LDH. Hovinen *et al.* (2016) found no differences in the NAGase activity between seasons. However, Nyman *et al.* (2014) reported that both LDH and NAGase activity were significant lower from September to November compared with December to April.

Leitner *et al.* (2004) studied the relationship between bacterial status and NAGase activity in 10 dairy goat herds. Bacterial status had a significant effect on NAGase activity. Barth *et al.* (2010) showed a similar relationship between NAGase activity and the infection status of goat milk samples. In contrast, Guliye *et al.* (2002) found that in camels there was no difference in NAGase activity in quarter milk samples that contained bacteria compared to those that did not. The type of bacteria in the udder did not influence NAGase activity (Guliye *et al.,* 2002). However, the differences in the NAGase and LDH activities in the study on bovine milk could depend on whether sampling was done from quarter milk or from composite milk (Hovine *et al.,* 2016)

The LDH and NAGase activities were more affected by cow factors such as days in milk, milk fat % and protein %, urea concentration, breed and milk yield, than was the SCC (Nyman *et al.,* 2014). The IMI status accounted for 23% of the variation in the SCC measurements whereas they explained only 7% and 2%, respectively, of the variation in NAGase and LDH (Nyman *et al.,* 2014). A high increase in SCC, NAGase and LDH in the monthly test milking results may be the result of an inflammatory response because of IMI instead of cow factors such as parity and days in lactation. Nyman *et al.* (2016) concluded that SCC was generally the most efficient way of identifying IMI-positive and IMI-negative dairy cows.

NAGase activity is also found in the blood (serum and in white blood cells); this enzyme can pass through the blood-milk barrier and be measured as NAGase from the epithelial cell cytoplasm of the mammary glands (Nagahata *et al.*, 1987; Kitchen *et al.,*1978). The NAGase enzymes that were obtained from the blood were reported by Kitchen *et al.* (1978) to be 5-15% of the total NAGase activity in cow milk. Piccinini *et al.* (2005) showed that the same amount of NAGase activity was observed in blood samples from both healthy dairy cows and dairy cows with a positive IMI status. However, the NAGase levels in quarter milk were significantly higher in unhealthy cows than healthy ones.

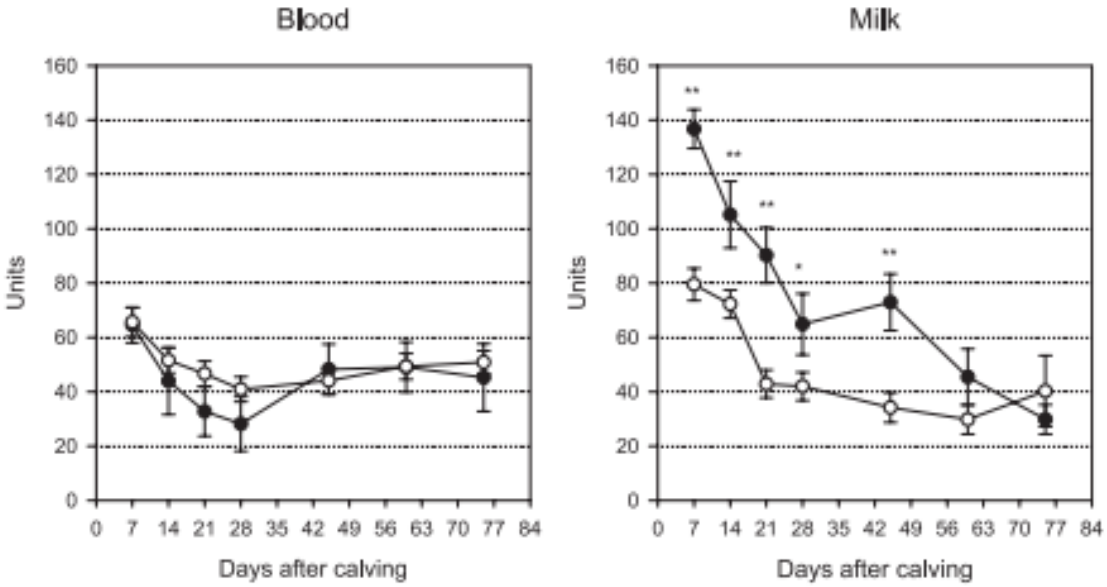


Figure 2: Distribution of NAGase in blood and milk from healthy (white) and IMI status (black) dairy cows (Piccinini et al., 2005).

Camel milk contains a high proportion of cell fragments without a nucleus. Abdurahman *et al.* (1992) compared camel milk, which contains a high number of this cell fragments, with goat milk, which also has a large amount of broken cell fragments in the milk. These cytoplasmic particles are a similar size to epithelial cells and could be mis-read as a high cell number. To avoid this error, a DNA-specific method should be used, such as a technique that counts cell nuclei (Nagy *et al.,* 2013). Abdurahman *et al.* (1992) considered that this could be a reason why camel milk has a higher NAGase activity than, for example, cow milk. A high milk yield of cows was shown to be associated with low LDH and NAGase activity (Nyman *et al.*, 2014).

Bacteria associated with mastitis

The type of bacteria infecting the udder of the camel will affect the SCC (Guliye *et al.,* 2002). The main mastitis bacteria for camels in Kenya are *S. aureus, E. coli, S. epidermidis* and *Str. agalactiae* (Toroitich *et al.,* 2017). Guliye *et al.* (2002) found that the SCC was highest if the udder quarter was infected with *S. aureus* and lowest if the infection was caused by *E. coli.* Although *S. aureus* are able to grow successfully between 8°C-46°C, the optimal growing temperature is 38.5°C (Medvedova *et al., 2*009).

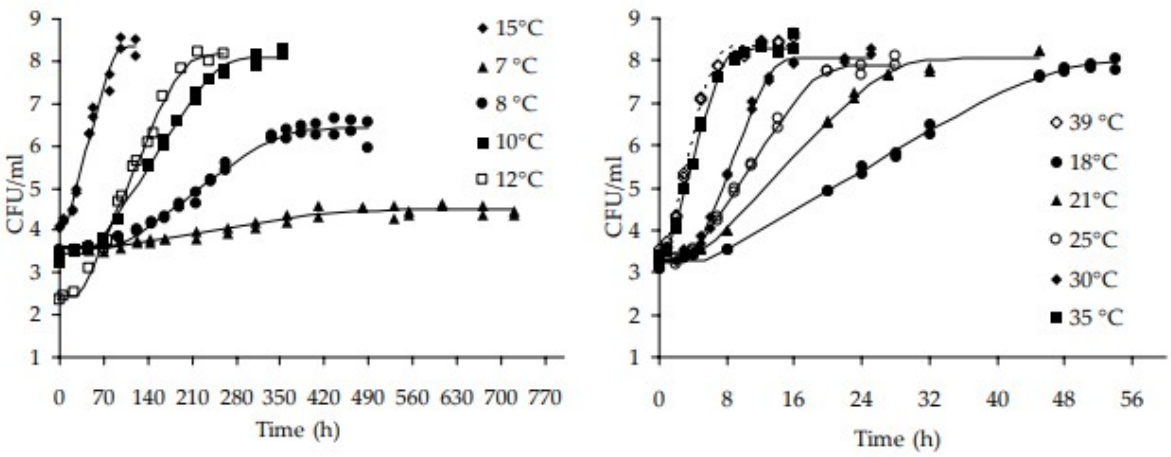


Figure 5: *The growth of Staphylococcus aureus 2064 in human milk at various incubation temperatures (Medvedova et al., 2009).*

Staphylococcus aureus is a highly contagious bacterium that is transmitted to other animals within the herd via milking equipment and the milkers´ hands; *S. aureus* could also infect the calf through the cow´s milk (Radostits *et al.,* 2007). In herds that have severe problems with the bacterium, *S. aureus* can also be found in wounds and on the skin of the hocks (Gustavsson, 2012). If there are wounds on the teats from cuts or from the cow stepping on the teat, the risk of *S. aureus* infection will be 93% compared with an undamaged teat where the risk for infection would be 53%. When a cow has *S. aureus* in its body, it is difficult to remove, even with treatment. The infections are often chronic and *S. aureus* is able to survive intracellularly (Radostits *et al.,* 2007).

Treatment

After a dairy cow with mastitis has been treated with antibiotics, the recovery period could last several weeks, as seen in a reduced milk yield and in LDH activity levels. Cows that had already been treated once for *S. aureus* mastitis had a 16% lower milk yield than control cows (Fogsgaard *et al*., 2015).

It is possible to treat mastitis bacteria, but the results vary. Younan (2002) reported that camel herdsmen were familiar with injectable drugs as a method of administering antibiotics, whereas they were not familiar with intramammary tubes. The teat canals of camels have a smaller dimension than those of cattle, which makes intramammary tubes unsuitable for camels. Intramammary treatment can cure up to 87.5% of *Str. agalactiae* mastitis infection in dairy cows if the cases were detected early, while the cure rate is reduced to 14.7% by a late detection and treatment (Hejlicek *et al.,* 1994). Younan, (2002) stated that the pastoralists camel keepers usually “only consider treatment” when mastitis is acute.

The conclusion from Younan (2002) is that the types of antibiotics and the dose rates used in the treatment of dairy cows could work well for camels. However, because of strong sunlight and high temperatures in the camel's living conditions, the stability of the drugs may be questionable and should be further investigated.

Temperature as an indicator of mastitis

The possibility of detecting mastitis early in dairy cows using a thermal camera has been investigated in several studies (Sathiyabarath *et al.,* 2016; Polat *et al.,* 2010; Hovinen *et al.,* 2008, Berry *et al.,* 2003). The advantage of using an infrared thermal camera is that it is a non-touch method and is rapid (Berry *et al.,* 2003).

Hovinen *et al.* (2008) introduced *E. coli* lipopolysaccharide into the left front udder quarters of six cows, while the right front udder quarters served as control quarters. The thermal camera could detect the induced mastitis by showing a 1-1.5°C increase in udder temperature. However, the clinical signs such as swelling and changes in the milk were seen before the temperature increased. Thus, their study did not support the theory of detecting mastitis at an early stage with a thermal camera (Hovinen *et al.,* 2008). In contrast, Scott *et al.* (2000) found that the udder surface temperature increased by 2.3°C when mastitis was induced with bacterial endotoxin.

The natural variation in udder temperature for dairy cows was investigated by Barry *et al.* (2003). Environmental temperature, together with the previous day´s udder temperatures, could together predict the expected udder temperature with a high precision. With this knowledge a baseline of expected udder temperature could be created. A difference in the expected udder temperature could indicate mastitis in the early stages. They also showed that the cow's udder temperature rose with exercise outside (Barry *et al.,* 2003).

The body temperature of a camel can have a 6°C variation, which is part of the camel´s way of conserving water (Wilson, 1998). Sathiyabarath *et al.* (2016) measured the body temperature of dairy cattle for 28 days, which was found to be 37.23 ± 0.08 °C, and the average udder skin surface temperature was 37.22 ± 0.04 °C. The cattle that were classified as having subclinical or clinical mastitis by CMT had an increase in udder temperature of 0.72 and 1.05°C, respectively. In another study, it was seen that the temperature of the cows´ udder surface was starting to increase up to 3 days before the onset of any clinical signs of mastitis (Hurnik *et al.,* 1984).

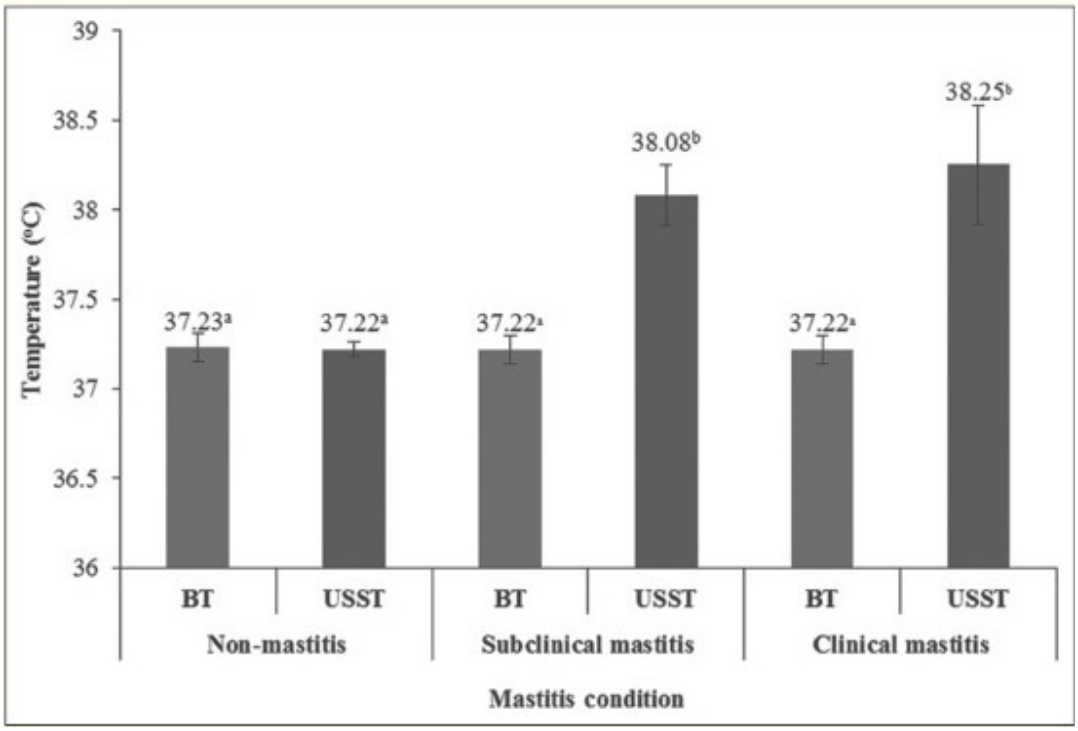


Figure 6*: The differences in udder skin surface temperature (USST) and body temperatures (BT) between non mastitis, subclinical mastitis and clinical mastitis in Holstein Friesian dairy cows (Sathiyabarathi et al., 2016)*

Apart from dairy cows, the technique of measuring udder surface temperature has been tested on dairy goats (Caruolo *et al.,* 1990) and sheep (Castro-Costa *et al.,* 2013; Mala *et al.,* 2009). Samara *et al.* (2013) studied the possibility of using an infrared thermometer on machine-milked dairy camels (*Camelus dromedarius*). The SCC and CMT were used for detection of subclinical mastitis. According to the CMT and SCC, the udder quarters with subclinical mastitis had a 1.42°C higher temperature than healthy quarters. Thus, they concluded that infrared thermography could be a method for early detection of subclinical mastitis (Samara *et al.,* 2013).

In a study by Polat *et al.* (2010), a correlation between CMT and udder skin temperature in dairy cows was observed. Negative CMT had a SCC mean value of 65 $x10^3$ cells/mL and a mean udder skin temperature of 33.23 °C, whereas a CMT of 3+ had a mean SCC of 3,653 $x10^3$ cells/mL and a mean udder skin temperature of 36.27°C. In their conclusion, they suggested that further research is needed to investigate how various cow factors, such as lactation month, age, milk production and feeding times, as well as environmental factors such as humidity and temperature, could interfere with a reliable infrared thermometer reading.

Materials and Method

Study area and herds

The study was conducted in the beginning of January to the end of February 2018, in the Laikipia district of Kenya. The study included six pastoralist camel (*Camelus dromedarius*) herds. The herds were chosen after consideration of the following: being similar holdings with similar feed and environments, the ease of access from the researchers´ basecamp, and the interest from the owners of the herds to participate in the study. The camels were kept under pastoralist management in a semi-arid area where feeding consisted of natural browsing. Due to time limitations and collaboration with camels and herdsmen, four of the six herds (A, B, C and D) were chosen for repeated measurements.

Picture 1: Maps of the locations of the sample area and the locations of the studied herds.

All the camels were held in traditional enclosures, or "bomas", overnight and at milking, whereas during the daytime they were herded in the surrounding area. The calves were separated from the group only at night, being allowed free access to their dams during the day. The milking frequency was 1-2 times a day at the time of sampling which was during the dry season.

No feed supplementation other than minerals and salt was added by the herdsmen. Most of the camels were of Somali breed with a few exceptions of Pakistan and Turkana breeds. The number of camels included in the study was 97, comprising 10 camels in Herd A, 40 in herd B, 11 in herd C, 20 in herd D, 6 in herd E and 10 in herd F. Herds A, B, D and F were watered once every second day, while herds C and E were watered once a week. In all the herds, the camels were treated against ticks.

Sample collection

The sample collection were made over a six week period. Herd visits were performed 30 times, varying from 3-7 herd visits per week. The visits were performed depending on the possibility of the herd to host the researchers and the accessibility of transportation. The visits were divided as equally as possible between the herds during the six weeks. The duration of

the visit was between 1.5 and 2.5 hours.
The camels were examined visually to confirm that their general health was good. Behavioural signs, such as normal activity, normal movement pattern, and showing curiosity, were noted as indicators of health.

California mastitis test

A visual inspection was made first to look for any signs of swelling, redness or injuries on the udder or abnormalities in the colour or texture of the milk that could indicate clinical mastitis. A CMT-test (Scandinavian scoring) was conducted at the first meeting with the camel herds at the camel boma, except for herds D and E, where the milk was collected in 10mL milk tubes and transported in a cooler to the lab at Mpala research center, where the CMT test was conducted 2h after milking. The herdsmen released a camel calf from the enclosure where the calves were kept during the night. Milk letdown was initiated by the stimulation of the calf's presence and attempting to suck. The final udder stimulation was done manually by the herdsman. When the milker felt that the milk had been let down in the udder cistern by the camel, one strike of milk was pulled out on the ground. Then the CMT paddle was filled with milk from one or two strikes, which was mixed with CMT liquid and stirred, and the result recorded.

Herd A had the smallest number of positive results of subclinical mastitis and was chosen as a control herd for milk percentage differences between paired udder quarters. The camels in herds B, C, and D that showed a positive CMT score were chosen to continue in the study if they had an opposite udder quarter that had a negative CMT test. In this way, the camels could serve as their own controls but could also be compared with the healthiest herd.

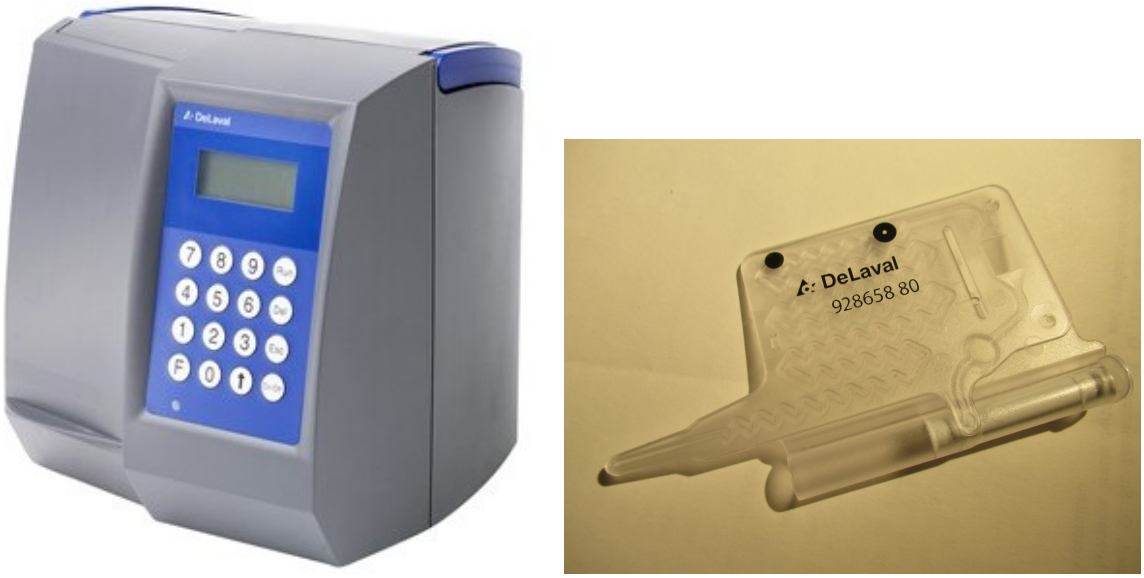
The results from the test were used to distinguish the camels with positive CMT readings (score ≥2) from the ones with negative CMT reaction (CMT score 1/ healthy). From these results, the camels that had quarter pairs (either front or hind) with one quarter with positive and the other quarter with negative CMT reaction, were chosen for continued sampling in the repeated measures study.

Picture 2: Camel milk mixture with CMT- contrast liquid on a CMT-paddle, right bottom corner indicates a strong positive reaction.

Somatic cell count

The SCC was analysed using Delaval´s Somatic Cell Counter (DCC). For herds A, B, and C, the milk from the CMT positive quarters and healthy opposite quarters was analysed the day after the CMT was performed due to time limitation. For herd D, the SCC was analysed the same day as the CMT was conducted.

The milk was collected in 10ml milk tubes during milking, when the milkers indicated that the camel had started to let the milk down. After milking, the milk was transported in a cooler to the lab at Mpala Research center. For camels that had produced milk samples showing a positive CMT reaction in one quarter and a negative CMT reaction in the opposite quarter, the samples were read using the DCC. The SCC was analysed for 179 milk samples.



Picture 3: The Delaval´s somatic cell counter DCC and the cassette used to draw the milk up in capillary tubes.

NAGase and LDH

N-acetyl-beta-D-glucosaminidase and Lactate dehydrogenase were analysed in the same samples as collected for the DCC. A total of 144 milk samples was frozen at -20°C in 4ml white bronopol tubes. The tubes were transported on dry ice from Nairobi, Kenya at the end of the study to Foulum Research Station Viborg, Denmark, where the enzymes levels were measured. NAGase activity was specified with an endpoint fluorometric assay according to Larsen (2010). LDH activity was analyzed with a fluorometric kinetic method according to Larsen (2010).

Milk yield

The milk measuring equipment was made from a 10 litre bucket with a lid. Inside the bucket,

4 x 1 litre plastic bottles were placed in mugs to stabilize them. The lid of the bucket had 4 openings in the top through which the top of the bottles protruded. Above the lid in the 4 bottle openings, 4 funnels were joined together. Using this construction, each udder quarter could be milked into separate bottles. Each bottle had a string tied around its neck. The string was used to hang the bottle on a scale to measure the milk in the bottle. The scale had an accuracy of two decimal places.

The bottle and string weighed together 0.03kg which was deducted from the total weight of the bottle and milk to give the milk yield measurement. The different colours on the funnels matched the string colour around the bottle necks to enable the milkers to keep track of which udder quarters had been sampled, to ensure that milk yield was measured and recorded correctly. After a bottle had been weighed, the contents were emptied into another container for the herdsmen to handle.

Picture 4: The milk bucket, allowing milk from each quarter to be collected separately for measurement of quarter milk yield.

Picture 5: The separate containers that contained the milk yield from the four udder quarters were weighed with a digital "hock" scale.

Temperature

The udder skin surface per quarter was measured repeatedly in herds A, B, C and D. The outside temperature was measured in the mornings on arrival and departure from the herds.

The temperature measurements were performed just before the calf started to stimulate the camel udder by sucking, just before the stimulus from the milkers started. The thermometer was a "Microlife NC150 non-contact thermometer", which used infrared light to measure the udder skin temperature. The thermometer was held at a distance of 3 cm from the udder surface skin. The temperature was shown for 3-4 seconds. The thermometer had a built-in memory for up to 30 temperature measurements. The camel´s four udder quarters were measured in a sequence. The temperature measurements were taken at the same milking as the milk yield was recorded, while the camel was milked. The thermometer was new and not calibrated during the study. Due to time limitations, the temperature was not measured the same day as the CMT.

Picture 6: The thermometer was held at a distance of 3 cm from the udder surface skin. "Microlife NC150 non-contact thermometer", (Picture Apotek Kronan)

Questionnaire

A questionnaire was created with three questions for the herdsman most responsible for the herd. The answers were recorded for 57 camels.

1. The number of calves the camel had produced
2. The lactation month of the camel
3. If the herdsman thought the camel produced a high, medium or low amount of milk

The answers for the third question were noted down as low = 1, medium = 2, high = 3.
The three answers were then compared with the camel´s highest CMT value from the four quarters.

The milking routines in the herds included in the study were observed for milking times, milking order, hygiene, equipment, storage and transport. Also the herdsmen were questioned about mastitis and subclinical mastitis, and their knowledge about the subject. Traditions and habits were discussed through conversations with the herdsmen and participants.

Statistical analyses

Descriptive statistics of distributions of SCC, NAGase, LDH and milk yield over CMT and all statistical analyses were performed in Stata (Release 15.1; College Station, TX, USA: StataCorp LP). Associations between CMT and SCC, NAGase, LDH and milk yield, as well as between SCC and milk yield, SCC and quarter placement, SCC and NAGase and SCC and LDH, were investigated using linear mixed effect regression models adjusting for repeated measurements within camel and herd.

Nagase and LDH values were compared with both CMT score and SCC from the same udder quarter.

Comparisons between milk yield in matched udder quarters were made using Wilcoxon-Sign

Rank Test. The quarter milk yield measurements taken in the 10-12 day period were then compared with the CMT result the camel had shown at the first CMT measurements. The milk yield measurements between CMT 1, 3, 4 and 5 were compared with CMT 1. The milk yield difference was then calculated in percentage. First, the CMT 1 udder quarters were compared with each other, to show the milk yield difference in percentage between two healthy udder quarters (CMT1), either “front front” or “hind hind”. Second, the milk yield from the quarter that was defined as healthy was compared with the milk yield from the other unhealthy quarters. The difference in milk yield from the compared udder quarters was calculated (%).- The differences in milk yield between quarters with CMT 1 and quarters with CMT 3, 4 and 5 were calculated to see how much the milk yield differs between udder quarters where one udder quarter is affected with subclinical mastitis. The results are shown in Figure 6.

An investigation was carried out to determine if the number of parities affected the subclinical mastitis status of the dam. A comparison between CMT and the lactation month was performed to investigate if CMT had any association with early, mid or late stage of lactation. The lactation month was compared with the highest CMT score of the camel´s four quarters. A comparison between CMT and the herdsman´s assessment of milk yield performance was done to see if the CMT had any association with the evaluated production level. The answering options for the herdsmen were low producer, middle producer or good producer, with the answers being given a number: low producer = 1, middle producer = 2, good producer = 3.

Results

Subclinical mastitis indicators and milk yield

CMT

In all, milk samples from 505 udder quarters from 97 camels in 6 herds were investigated using CMT; the results are presented in table 1. The median CMT score was 1 (inter quartile range (IQR) 1 – 1). There was at least one camel in each herd that had a quarter with CMT≥3. The herd prevalence of camels with CMT≥3 for the six herds A, B, C, D, E and F was 10%, 14.7%, 20.9%, 22,5%, 37.5% and 35.3%. The prevalence of CMT ≥3 on udder quarter level was 2.5%, 7.1%, 14%, 17.5%, 17.6% and 25%.

Table 1: Distribution of Californian mastitis test scores (number of quarter milk samples (%) and distribution, median (inter quartile range (IQR)) and mean values (standard deviation (SD) of (SCC, NAGase, LDH and milk yield) in quarter milk samples and quarter milk yield for each CMT score.

CMT score	1	2	3	4	5
n	389 (77%)	47 (9%)	29 (6%)	25 (5%)	15 (3%)
SCC,*1000cells/ml					
n	72	22	21	17	12
Median (IQR)	48.5 (23.5 – 112)	206 (126 – 470)	434 (331 – 888)	1370 (923 – 1998)	4561 (3929 – 5551)
Mean (SD)	234 (820)	303 (227)	702 (668)	1627 (1180)	4501 (1148)
NAGase					
n	58	14	17	13	7
Median (IQR)	16.5 (13.6 – 21.2)	20.6 (17.0 – 27.7)	19.2 (16.0 – 23.6)	30.4 (21.7 – 45.9)	42.1 (29.5 – 111.4)
Mean (SD)	18.5 (8.1)	21.8 (6.4)	24.6 (21.5)	38.4 (23.2)	59.5 (43.0)
LDH					
n	58	14	17	13	7
Median (IQR)	8.8 (7.2 – 12.6)	12.6 (10.5 – 14.6)	12.7 (10.6 – 16.4)	25.7 (21.1 – 33.3)	64.6 (35.1 – 81.5)
Mean (SD)	10.4 (5.3)	14.2 (6.7)	14.2 (6.7)	29.5 (16.0)	62.3 (23.1)
Milk yield					
n (not including milk yield=0.01)	60	9	9	6	2
Median (IQR)	0.33 (0.02 – 0.42)	0.24 (0.22 – 0.31)	0.32 (0.12 – 0.38)	0.15 (0.14 – 0.17)	0.11 (0.06 – 0.17)
Mean (SD)	0.33 (0.15)	0.25 (0.06)	0.31 (0.19)	0.19 (0.14)	0.11 (0.08)

Somatic cell count and association with CMT

The SCC was analyzed in 144 udder quarter milk samples. The median SCC was 162,000 cells/mL (IQR: 48,500- 888,500 cells/mL); the mean SCC was 832,800 cells/mL (SD:1446 200 cells/mL). There was a linear association between SCC and CMT where SCC was significantly higher with increasing CMT for all comparisons of CMT classes ($p<0.01$) (Figure 1).

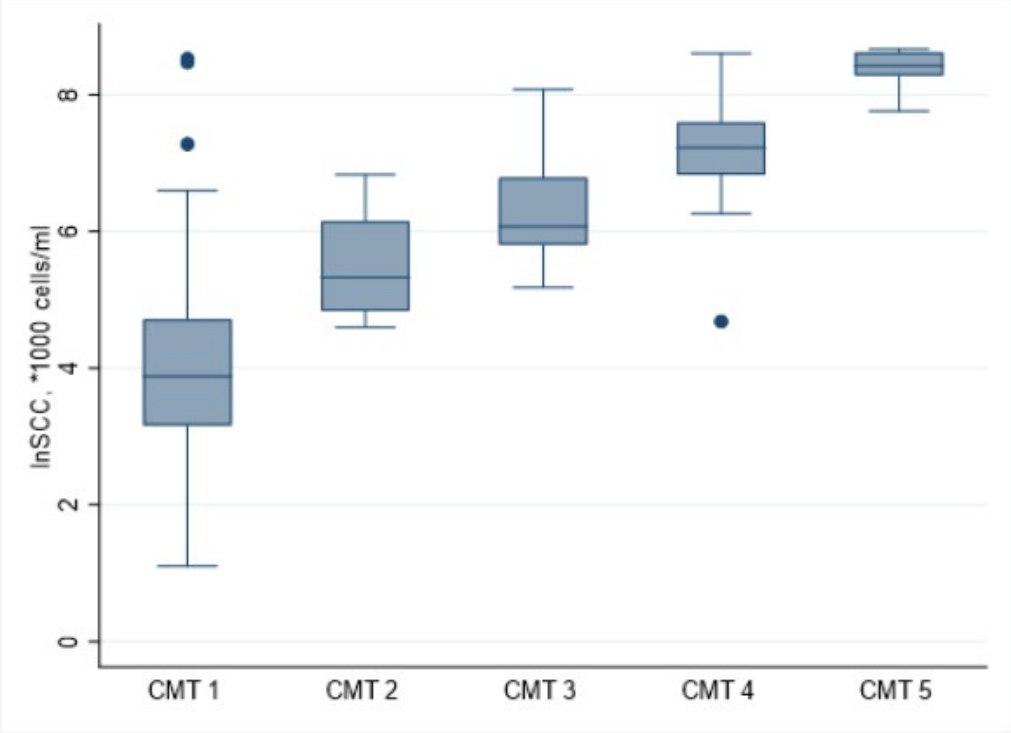


Figure 1: Box-and-whisker plot of somatic cell count (log converted ic to lnSCC) levels in milk samples with CMT scores 1-5.

A significant association was seen between the udder quarter placement and SCC, being lowest in the right front quarter and highest in the right hind quarter.

Table 2: The relationship between the position of udders quarters and SCC.

Teat position	Observation	Mean (SD)	Min	Max
LF	40	997 (1527)	26	5461
LH	33	700 (1307)	10	5549
RF	39	623 (1242)	4	5784
RH	32	1021 (1708)	3	5561

NAGase and LDH and associations with CMT

The enzymes NAGase and LDH were analysed in 111 samples. The median and mean NAGase activity was 19.6 U/L (IQR:15.3 – 26.3) and 24.9±19.8, respectively. The median and mean activity of LDH was 12.0 U/L(IQR: 8.5 – 16.8) and 17.1±16.1, respectively. There was a significant association between the NAGase levels and CMT with significantly higher NAGase levels in milk with CMT 4 and 5 compared to milk with lower CMT scores (p<0.05) Figure 2. There was also a significant association between LDH levels and CMT with significant lower LDH levels in milk with CMT 1 compared to all other CMT categories (p<0.001) and when comparing all other CMT categories with each other (p<0.001), except for CMT 2 compared with CMT 3 (p=0.59) (Figure 3)

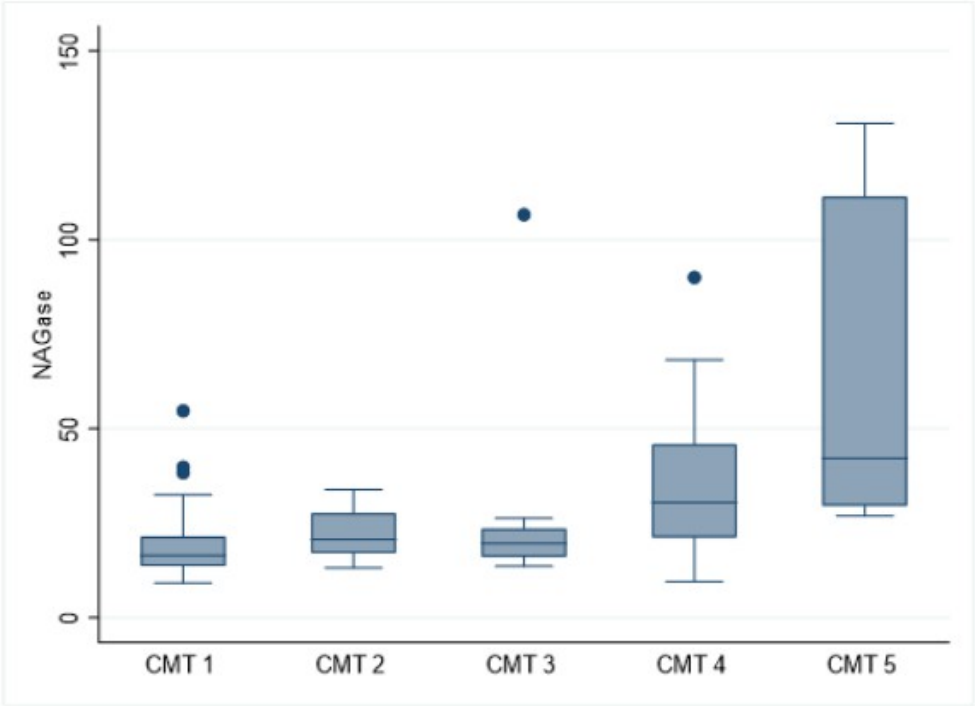


Figure 2: Box-and-whisker plots of N-acetyl-beta-D-glucosaminidase (NAGase) enzyme levels for quarter milk samples with CMT scores 1 – 5.

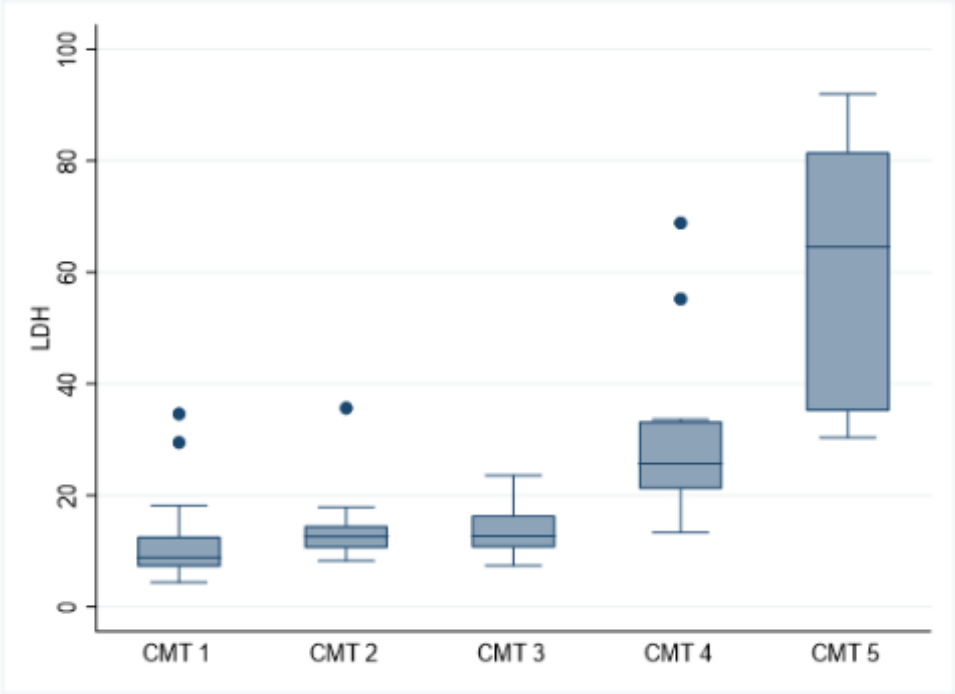


Figure 3: Box-and-whisker plots of Lactate dehydrogenase (LDH) enzyme levels in milk samples with CMT scores 1 – 5.

There was also a significant relationship between NAGase and LDH levels and SCC. (Figure 4), where the NAGase and LDH levels were higher with increasing cell count.

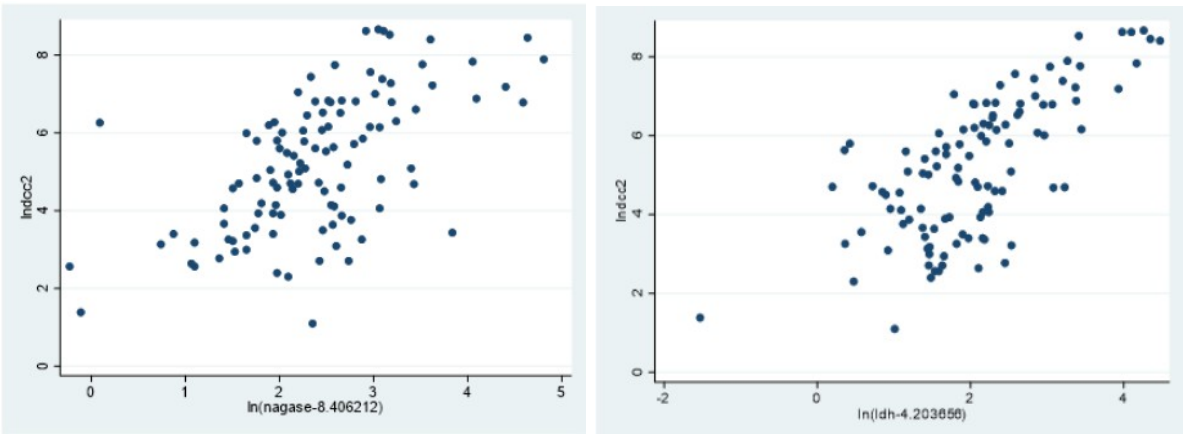


Figure 4: Relationship between SCC and enzymes NAGase (left) and LDH (right).

Milk yield

Milk yield was measured in 648 udder quarters. The median milk yield was 0.38 kg (IQR: 0.25 – 0.51 kg) and the mean was 0.39 ±0.20 kg. The highest milk yield measured in one udder quarter was 0.98kg. There was no significant association between milk yield and CMT (Figure 5) or between milk yield and SCC.

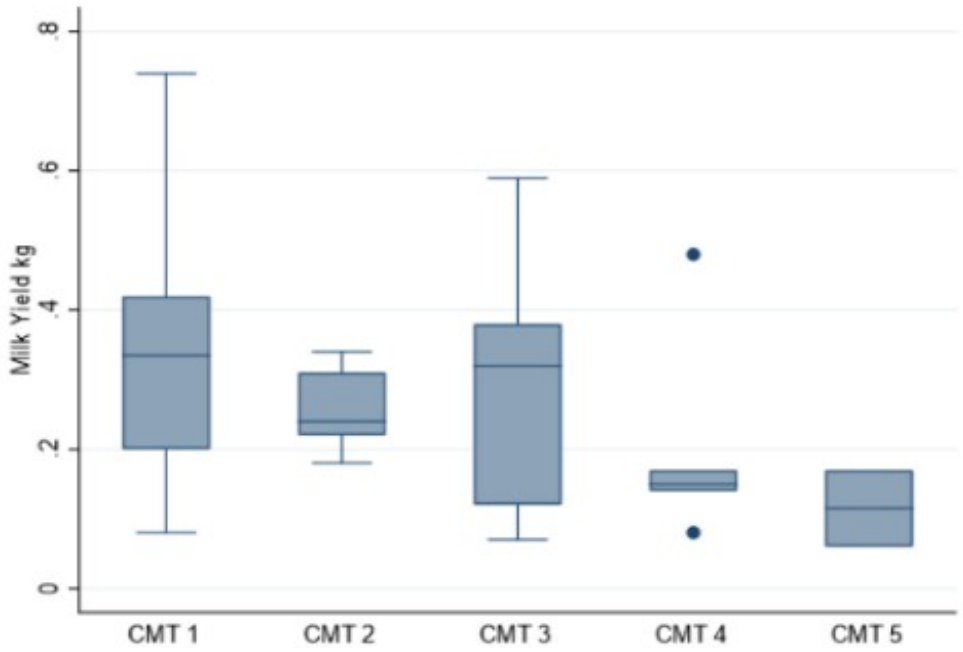


Figure 5: Box-and-whisker plot of milk yield in milk samples with CMT score 1-5.

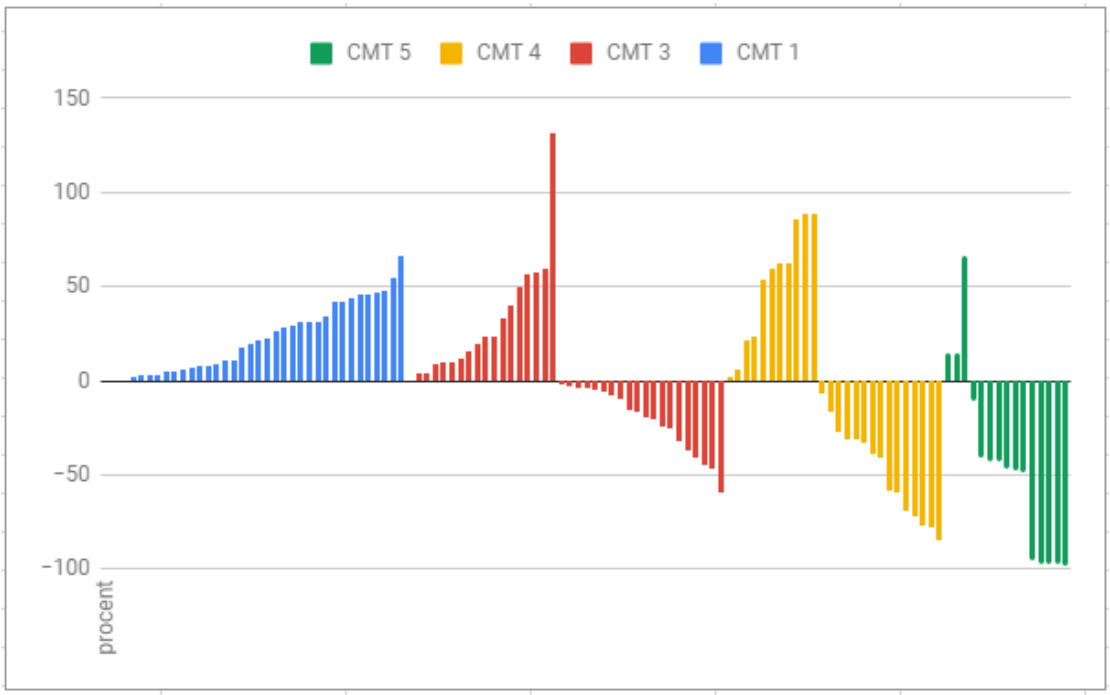


Figure 6: The difference in milk yields between front- front or hind-hind teats is shown in Figure 6, where 0% represents no difference in milk yield between the compared teats. The blue CMT 1 category is the percentage difference in yield between two healthy quarters. The negative value for quarters with CMT 2, 3, 4 and 5 indicates the reduction in milk yield from the subclinical mastitis quarter t compared to the healthy quarter (CMT 1), whereas a positive value for quarters with CMT 2, 3, 4 and 5 indicates that the subclinical mastitis quarter is producing more than the healthy quarter.

With a CMT 5 in one of its paired udder quarters, the milk yield was, on average, 44.7% (maximum 66% and minimum 0%) less in the quarter with subclinical mastitis (n=15). With a CMT 4 in one of its paired udder quarters, there was, on average, 6.6% (maximum 131% and minimum -59%) less milk in the quarter with subclinical mastitis (n=26). For a CMT 3 in one of the paired udder quarters, there was, on average, 3.5% (maximum 89% and minimum -85%) more milk in the quarter with subclinical mastitis (n=38). The average difference in milk yield between two healthy quarters (CMT 1) was 22.4% (maximum 66% and minimum -98%) (n=36).

Due to time limitation, the CMT measurements were not performed with each milk yield measurement but were conducted at the first herd visit in all herds. In herd A the next CMT were conducted after 7 days and all the udder quarters that still showed CMT 1 were then counted as CMT 1 on the two subsequent days. In herd B the next CMT measurements were conducted after 12 days and in herd C after 10 days. Herd D did not have a second CMT measurement, due to time limitation. The two CMT results showed that, in most cases, if a camel had CMT 3 or more at first measurement, the CMT did not decrease during the 10-12 day period.

Temperature

The environmental temperature varied from 17°C to 23°C and was not taken into account when handling the udder skin temperature data. There was no clear pattern regarding the temperature and the milk yield (Figure 7)

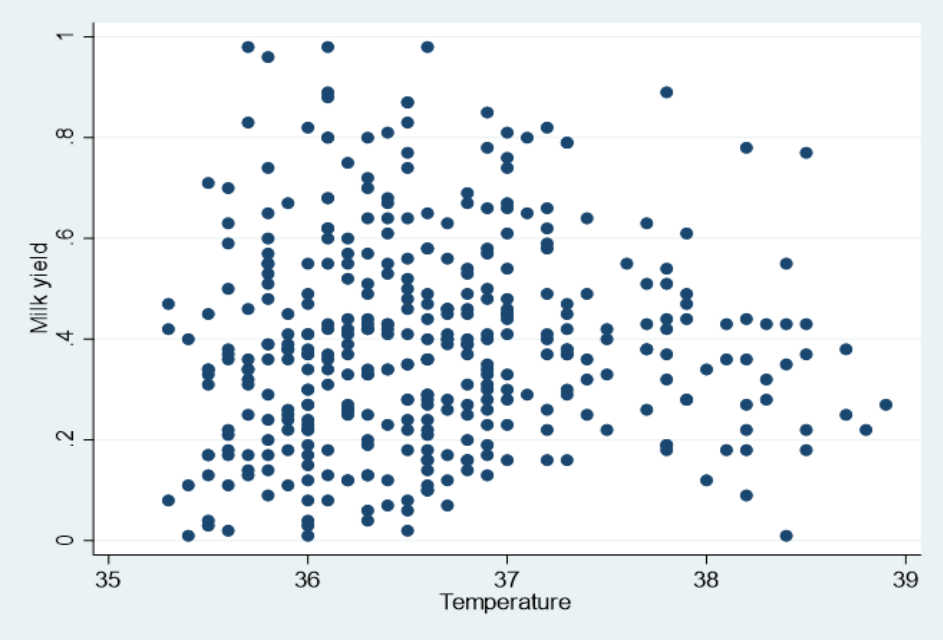


Figure 7: Box-and-whisker plot of the association between udder skin temperature in udder quarters and milk yield.

Questionnaire

The mean number of calves that the camels had produced, the mean lactation month in which the sampling occurred, and the means of the herdsman´s evaluation of the camel´s production (Low =1, Medium =2 or Good =3), compared to the camel´s highest CMT score of the four quarters are shown in Figure 8.

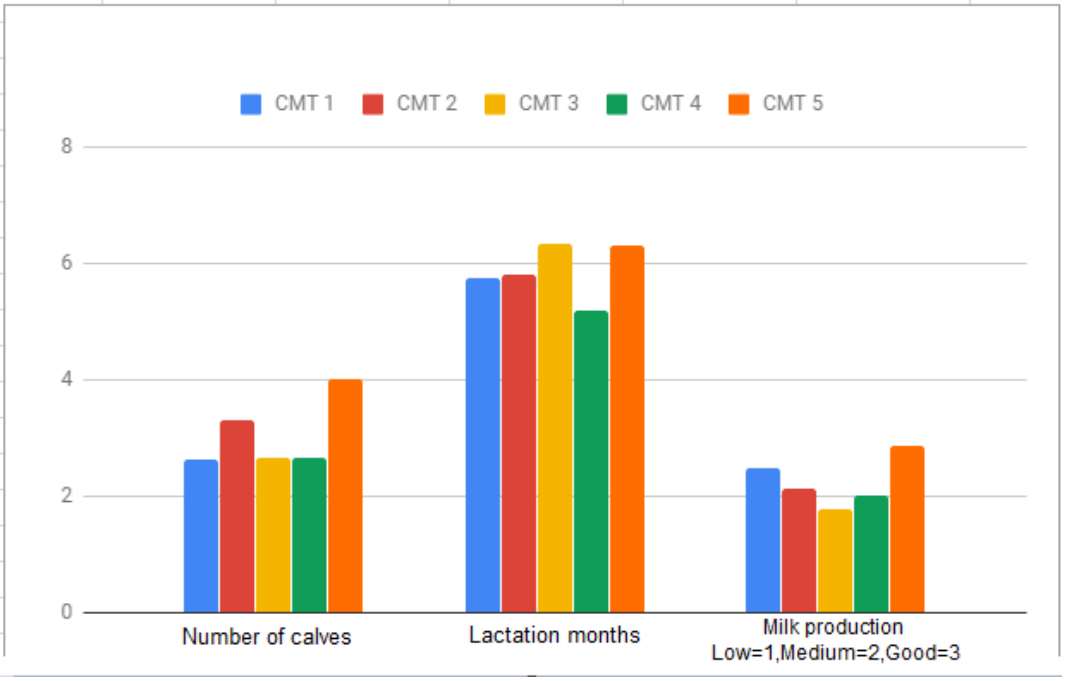


Figure 8: The average number of calves, average lactation month and average milk production for camels with different CMT scores.

Figure 9 shows the distribution of CMT related to the number of calves, the lactation month and the herdsman´s own evaluation of the camel´s production, low, medium or good (values indicated on the y-axis). Compared to Figure 8 showing the mean score of the answers, Figure 9 shows the herdsmen´s responses and the number of camels that were included in each CMT group. The number of camels in each CMT group shows how common that CMT score was for that question.

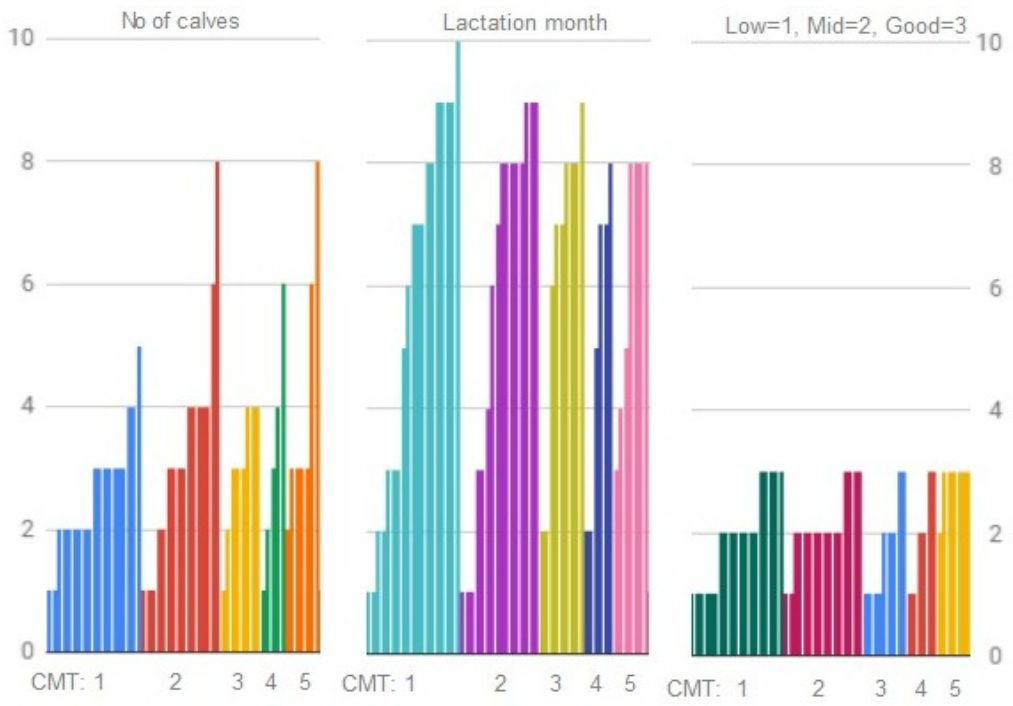


Figure 9: Relationships between the number of calves, the lactation month, and the herdsman´s own evaluation of the camel´s production. Each category shows the number of camels that was included in the CMT group.

The herds that were included in the study was seen to have inconsistent milking and hygiene routines. There was a lack of milking order, milking time, sanitizing of equipment, or the possibility to cool the milk. In addition, four of five herds used disposable plastic containers to store and transport milk, instead of metal containers. There was a lack of knowledge of methods for controlling the occurrence of subclinical mastitis. Also some tribes have a tradition of not selling or slaughtering the female camels, resulting in retention of camels that were affected with mastitis or with blind teats.

Discussion

Subclinical mastitis is a major challenge to camel dairy production for pastoralists in Kenya. The aim of this study was to investigate if CMT could be of use to identify udder quarters with subclinical mastitis and if there were any associations between subclinical mastitis and quarter milk yields in dromedaries in pastoralist herds in Kenya. There was a significant relationship between CMT with SCC, NAGase and LDH, showing that CMT would be a sufficiently accurate tool to be used in the field to detect camels with subclinical mastitis. The SCC had the strongest connection with CMT score, compared to NAGase and LDH. This is logical because CMT is a way to measure SCC. Subclinical mastitis was measured by CMT scoring as well as with the inflammatory markers SCC, NAGase and LDH. However, CMT 1, 4 and 5 had the most distinct linear correlation with these markers, whereas CMT 2 was harder to distinguish by observation, perhaps because it represent a transition zone. Udder skin temperature did not have any relationship with milk yield.

The SCC for *Camelus dromedarius* investigated in the present study correspond to the SCC for cows, according to both international CMT and Scandinavian standards, as shown in the literature review (Goncalves, 2017). However, the interquartile range showed a slightly lower median interval for camel (<112,000 cells) than for the SCC interval for cows (<200,000). Hamed *et al.* (2010) did see that camel milk had a lower mean value of SCC than dairy cows when both animals were studied in two groups with high and low SCC. It appears that CMT is a good marker for subclinical mastitis.

The SCC was lowest in the right front quarter and highest in the right hind quarter. The part of the udder that had the highest prevalence of mastitis was significant. The percentage difference in milk yield between front and hind quarters (40%-60%) did not explain the SCC difference between front and hind quarters.

Both the enzymes NAGase and LDH are significantly associated with SCC, in agreement with Hovinen *et al.* (2016), who stated that NAGase could be used with an accuracy of 85-99% for subclinical and clinical mastitis. Also Nyman *et al.* (2014) showed that NAGase and LDH are higher in mastitic cows than in cows without any intramammary infections. Earlier observations of the value of NAGase and LDH in detecting mastitis were made by Bogin *et al.* (1973) and Kitchen *et al.* (1978). The median(IQR) values of NAGase and LDH in healthy udders, in the present study were 16.5U/L (13.6-21.2), and 8.8U/L (7.2-12.6). Mean values for NAGase and LDH in goats for early, mid and late lactation were 1.9, 1.3 and 4.5 U/L, and 3.2, 3.3 and 7.7 U/L, respectively (Persson *et al.,* 2014). Mean (SD) values for cows were 4.69 (2.21) U/L and 1.73 (1.34) U/L (Nyman *et al.,* 2014). The enzyme levels for camels are higher than for goats and cows, but it was not possible to determine if this depends on the type of animal or the study environment. Both NAGase and LDH were significantly associated with CMT scoring, indicating that CMT would be a good marker for subclinical mastitis in the dromedary camel. However, there were too few NAGase and LDH measurements to see any association with milk yield.

The results of the milk yield from the camels were in agreement with earlier studies by Onjoro *et al.* (2006) and KARI. There was no significant association between milk yield and CMT scores or between yield and SCC. The percentage difference in milk yield between CMT 4 or CMT 5 quarter and a CMT negative quarter indicated that a high CMT is associated with a lower milk yield. That CMT 3 quarters produced more milk than their paired healthy quarters is surprising. However, the average differences in milk yield between

two healthy quarters (CMT 1) was 22.4%, indicating that, in general, there is a big difference in milk yield between quarters. Forster *et al.* (2010) used the same method for dairy cows to estimate percentage milk lost due to CMT 3 or more, in paired quarters. The results in Forster ´s study showed a decrease in milk of 9.0% for CMT 2, 19.5% for CMT 3, 31.8% for CMT 4 and 43.4% for CMT 5. The percentage loss for CMT 5 in the present study is in agreement with the results of Forster *et al.* (2010). An explanation of the increase in milk yield for CMT 3 could be the large variation in milk yield between the paired quarters. To our knowledge, studies on milk yield differences between paired healthy quarters in camels have not been done before.

By knowing that CMT 4 and 5 exist in 8% of all 505 udder quarters that were tested in this study and that milk yield decreases in average 6.6% (CMT 4) and 44.7% (CMT 5), the impact on the pastoralists´ economy can be considered. The average milk yield is 0.39 kg ~ 4 dl. The herdsmen are paid approximately 100 Ksh per liter of raw milk, or 40 Ksh for 4 dl, which is approximately 4 SEK. A camel´s lactation period is 11.8 months, on average, but could last up to 18 months. Thus, on average a herdsman can lose from 960 Ksh (96 SEK) to 6500 Ksh (650 SEK) per udder quarter that contain subclinical mastitis every year. A camel can produce up to 12 calves which means 12 lactation periods. Also a lost quarter due to mastitis could mean 14,600 Ksh (1,460 SEK) lost income every year. These numbers are based on the milk yield for camels in this study in the dry season, with one milking per day. A greater loss in income would be found if the calculation is based on the milk yield during the wet season, which could be double that in the dry season. The minimum wage allowed in Kenya according to *Tradingeconomics* is 13,572 Ksh (1,357 SEK).

One observation that could explain the differences in milk yields within camels could be the milking performance of the herdsmen. The milk bucket that was constructed for this study worked well. After a test-run, replacing the funnels with ones which had a more conical shape allowed the milk to be collected without any waste. The herdsmen understood the system well, and were able to milk one teat into each funnel. The milking was also monitored by the responsible supervisor. The herdsmen were milking two by two, with one herdsman on each side of the camel. One herdsman helped to stimulate the camel and was ready with the bucket, while the second herdsman was focused on the calf and the camel herd. The calf was allowed only to suck enough to stimulate milk let–down without getting any milk; the second herdsman watched it carefully and, while milking, he also had to push the calf away from the udder continually. Due to this problem, milking was not done in an equivalent manner on both sides of the camel. Another factor involved in uneven milking of the camel´s right and left side, is the herdsmen´s habit of chewing “Mirra”. “Mirra” is a leaf that contains a stimulant drug that could affect the engagement of the chewer on his task.

The udder skin temperature measurements in the study did not show any clear pattern regarding an association with milk yield. In previous studies with cows (Sathiybarath *et al.,* 2016; Polat *et al.,* 2010; Hovinen *et al.,* 2008), goats (Caruolo et al., 1990) and sheep (Castro-Costa *et al.,* 2013; Mala *et al.,* 2009), the infrared thermometer was shown to be useful as an early indicator of mastitis. Samara *et al.* (2013) found that camels with subclinical mastitis had an udder surface temperature 1.42°C higher than a healthy camel. The result from the present study did not show that the infrared thermometer ”Microlife NC150 non-contact thermometer” was a good indicator of subclinical mastitis. Due to time limitations, the CMT could not be measured the same day as udder temperature, and milk yield did not show any association with temperature. This could be due to the low number of animals in the study or

the time of the measurements. If a long-term study were to be performed where the udder skin temperatures could be monitored and controlled within the same camel, it is possible that a different result could be obtained. Further investigations are needed.

The questionnaire provided additional knowledge to the CMT, such that the camels with a higher parity number also had a numerical higher CMT score. This could be due to both a physiological variation well known in cows and/or a difference in the prevalence of mastitis. Ahmad *et al.* (2012) showed that parity number had a significant relationship to mastitis in camels, while Obied *et al.* (1996) did not find any physiological difference in SCC with increasing lactation number in mastitis-free camels

The result of the CMT scoring during the lactation period in the present study, gives unclear information; a larger number of camels would be preferable. Ahmad *et al.* (2012) found a significant relationship between mastitis and lactation stage in camels while Obied *et al.* (1996) did not observe any relationship between SCC and stage of lactation in healthy camels. From the herdsman´s own evaluation about the camel´s level of production in the present study, the camels that were said to be high milkers also had a high CMT score. However, the udder quarter samples showed a relationship between low SCCs and a high milk production, indicating that the perception of the herders might be wrong, or possibly that the camels with CMT 5 in one udder quarter produce more milk at the whole udder level. High-producing dairy cows are often more prone to mastitis and perhaps it is the same for camels.

The main difference in milking practice observed between the herds was the use of hand sanitizer in herd A. The herdsmen were using the sanitizer between milking different camels, which likely prevented transmission of bacteria from one camel to the next, which could explain the better udder health in this herd. There are several obstacles to including additional hygienic routines in the milking process. First, an understanding of why hygiene is important to the dairy market and for the individual camel is needed, to be able to prevent subclinical mastitis as well as to treat clinical cases. Knowledge of how bacteria are transmitted between the animals and herds is necessary, and the people who handle the animals need to be educated about hygiene. The average highest and lowest temperatures in the Laikipia district in Kenya are between 25-26°C and 9-10°C, respectively (Weather statistics YR 2018). The temperature at which *S. aureus* 2064 grows is 8-46 °C (Medvedova *et al.,* 2009). This means that there is always a beneficial climate for growth of bacteria in milk. Due to the tradition of not selling or slaughtering female camels, those animals with very severe or chronic mastitis problems remain in the herd and could transmit bacteria to the rest of the animals for many years.

A suggestion in order to improve the hygiene of camel milk would be for the dairies to place a higher demand on low SCC in the delivered milk, as already done in many countries. A common way today in at least the smaller dairies in Kenya is that the dairy has a person who smells and tastes the milk to decide if it should be approved or not. Introduction of a payment-based system would encourage low SCC because of its higher value. The owners of the herds would be interested in gaining as much profit as possible, and therefore take action against high SCC. A CMT-kit with a paddle is a very cheap and easy way to check the subclinical mastitis situation in the camel herd. Establishing a milking order would be beneficial, with the healthiest animals being milked first and those with higher CMT last.

Herd A was the herd with lowest prevalence of subclinical mastitis and also the only herd

where the milkers used hand sanitizer frequently. No studies have been done to compare herds with and without hand sanitizers yet, but it could be an interesting continuation to the present study. If use of the hand sanitizer is related to a lower cell count, it would be a cheap and easy way of preventing bacteria spreading between the camels on the hands of the milkers. Another improvement that could be done is to use metal containers instead of disposable plastic containers, since metal would be more hygienic and easier to clean.

The environment around the camels is beneficial for control of subclinical mastitis. The camel´s udder is attached high up between its hind legs. During the study, it was observed that the camels´ udders did not touch the ground. The camels´ faeces were dry; the udder was not contaminated with either faeces or urine. The risk for the camel to accidentally step on the teats was unlikely, because of the height of the udder. All these environmental risk factors are ways for mastitis bacteria to enter the udder, but were observed to be low during the study.

Conclusion

The results presented in this study show reality in the arid and semi-arid parts of Laikipia district in Kenya, rather than in intensively farmed dairy camels. The inflammatory markers SCC, NAGase, LDH and CMT are good markers for subclinical mastitis for *Camelus dromedaries,* although udder skin temperature did not work well as a marker in this study. The milk yield did not show any significant relationship with CMT or with SCC. However, the percentage difference in milk yield between the paired udder quarters showed that a high CMT score could be associated with a decrease in milk yield.

As CMT is an easy and cheap way of detecting udder quarters with subclinical mastitis,that could be used in pastoral camel herds to improve udder health and camel milk quality in Kenya.

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