Milking management of dairy buffaloes

C.S.Thomas
Department of Animal Nutrition and Management
Uppsala

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Abstract

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The aim of the thesis was to investigate some of the underlying principles for milking management of Murrah buffaloes. The study was carried out through five experiments, four on an experimental farm in India and one as a field survey.

From the results it was observed that the total cisternal area and cisternal fraction of milk in Murrah buffaloes was smaller than in dairy cattle, sheep and goat. Teats were longer and thicker and had longer teat canals than reported in dairy cattle. Teats were flaccid and empty prior to milk ejection due to the small cisternal fraction of milk, while during milk ejection there was a remarkable increase in teat dimensions and cisternal area. Commencement of milk flow during machine milking took longer without pre-stimulation than in the other dairy species due to the small cisternal fraction of milk. The combined stimulation of feeding during milking and manual pre-stimulation resulted in a faster and more pronounced release of oxytocin, prolactin and cortisol compared to milking with only manual pre-stimulation and no pre-stimulation. Small changes in the milking routines adversely effect oxytocin release, milk ejection and complete removal of milk, which was also reflected in the stripping yield and fat percent in stripping milk. The daily rhythm of maintenance behaviour of buffaloes with mechanisation of some farm chores was comparable to those without mechanisation. However, increased access to roughage increased the resting behaviour and the animals ate longer during the night as well. The milking management systems and the herd size had an impact on the udder health status of buffaloes. The prevalence of mastitis was lower in the small buffalo farms where buffaloes were stimulated by calf suckling and hand milked compared to large farms where the buffaloes were manually pre-stimulated and hand milked or machine milked.

In conclusion, during machine milking of buffaloes owing to the small cisternal milk fraction, attaching the milking cluster prior to milk ejection could lead to higher teat penetration in the teat cup and milking on empty teats. To prevent a possible irritation and stress due to machine milking without milk flow at high vacuum, milk removal should be synchronised with milk ejection. It is thus necessary to improve machine milking settings for buffaloes and to develop ISO standards optimised to suit the anatomical and physiological characteristics of buffaloes. Training of milkers and milking machine users is also important for improving the udder health and welfare of buffaloes.

Keywords. Machine milking, milk ejection, pre-stimulation, oxytocin, udder health, maintenance behaviour, cisternal size.

Author´s address: Chirathalattu S. Thomas, Sr. no.80, Bung. no.1, Sudarshan Nagar, Pimple gurav, Pune 411027, India. *e-mail:tomninan@yahoo.com*

To my dear India the love of my heart - And to our beloved Ammachi and Appachen (My Parents) who served India and became poor to make many rich.

Where the mind is without fear and the head is held high;
Where knowledge is free;
Where the world has not been broken up into fragments by narrow domestic walls;
Where words come out from the depth of truth;
Where tireless striving stretches its arms towards perfection;
Where the clear stream of reason has not lost its way
into the dreary desert sand of dead habit;
Where the mind is led forward by thee into ever-widening thought and action--
Into that heaven of freedom, my Father, let my country awake.

Rabindranath Tagore
(From Geetanjali, 1910)

Contents

Preface

The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Thomas, C.S., Svennersten-Sjaunja, K., Bhosrekar, M.R. and Bruckmaier, R.M. 2004. Mammary cisternal size, cisternal milk and milk ejection in Murrah buffaloes. *Journal of Dairy Research (In press)*

II. Thomas, C.S., Bruckmaier, R.M., Bhosrekar, M.R. and Svennersten-Sjaunja, K. 2004. Short term influences of different tactile stimulations on machine milking characteristics in Murrah buffaloes. *(Submitted).*

III. Thomas, C.S., Bruckmaier, R.M., Östensson, K. and, Svennersten-Sjaunja, K. 2004. Effect of different milking routines on milking-related release of the hormones oxytocin, prolactin and cortisol and on milk yield and milking performance in Murrah buffaloes. *Journal of Dairy Research (In press).*

IV. Thomas, C.S., Nordstrom, J., Svennersten-Sjaunja, K. and Wiktorsson, H. 2004. Maintenance behaviour, behaviour during milking and milking characteristics in Murrah buffaloes during two feeding regimes. *(Submitted).*

V. Thomas, C.S., Nimmervoll, H., Boije, C., Svennersten-Sjaunja, K., Lundeheim, N. and , Östensson, K. 2004. Occurrence of subclinical mastitis in buffaloes in different herd sizes and milking management systems *(Submitted).*

Paper I and III have been used with the kind permission of the journal concerned.

Introduction

World dairy buffalo production

World milk production has doubled in the last decades and it is noteworthy that in the last years, buffaloes have been supplying about 12% of the world milk production. India and Pakistan have been producing 60 and 30%, respectively, of the world's buffalo milk. In both these countries buffalo milk contributes to 55 and 75% of the total milk produced (FAO, 2004).

Dairy buffalo production has been a tradition in many parts of the world like the Caucasian countries, Asia, and Egypt where fresh buffalo milk, dahi (cultured sour milk), ghee (butter oil), and yoghurt are popular. In Italy the dairy buffalo industry is flourishing on account of the popularity of buffalo mozzarella cheese. Because of the market for mozzarella, buffalo farming is a profitable enterprise and is carried out in an organised manner with modern tools. In South American countries like Brazil and Argentina, buffaloes are reared for both milk and meat. In recent years, buffalo milk and milk products, especially mozzarella cheese have become immensely popular and dairy buffalo production has found its way into non traditional areas with the number of buffalo farms mushrooming in the UK (*e.g.* http://www.buffalomilk.co.uk; 2004) and even in the USA. In India as well as Pakistan, in the vicinity of all the major cities like Mumbai, Calcutta, and Karachi, one can find a large number of buffalo farms of varying herd sizes. In Mumbai City alone, there are more than 200,000 buffaloes inside the main city and probably another 100,000 in the suburban areas. Some of these farms have herd sizes over 1000 buffaloes and on an average the herd size is above 100 buffaloes (personal communication, Mr. Subhash Vidya, General Manager, Dairy Owners League and Co-operative Society, DOLCOS, Mumbai). Large-scale dairy buffalo production is a greater reality in India and Pakistan than anywhere else in the world.

India produces more than 84 million tons of milk, with 80% of the being milk produced by small backyard farms with herds of 2 to 8 animals, usually both buffaloes and cows together. These micro enterprises are operated by an estimated 11 million farmers located in remote villages who are members of 96,000-odd village dairy co-operatives. The producers, mainly landless labourers with underprivileged families, enjoy a relatively steady income through the sale of surplus milk. This income is vital to their well being and economic security (Manorama India Yearbook, 1998; IFCN, 2003).

The domestic water buffalo

The domestic water buffalo (*Bubalus bubalis*) is from the *Bovidae* family, sub-family *Bovinae*, genus *bubalis* and species *arni* or wild Indian buffalo. The animals are classified into two distinct classes as swamp and river buffalo. The water buffalo has many anatomical and physiological similarities with the other species in this family. Cattle have 60 pairs of chromosomes, river buffaloes have 50 and swamp buffaloes have 48. While the two types of buffaloes can be mated to produce a fertile offspring having 49 pairs of chromosomes, buffaloes cannot be successfully mated with any others in the *Bovidae* family (for review see Mahadevan, 1992).

The swamp buffaloes are found in the Indian subcontinent and throughout Southeast Asia and China. The name 'swamp' has probably arisen from the fact that they prefer to wallow in stagnant water pools and mud holes. These buffaloes produce relatively small quantities of milk, 1 to 2 kg per day and they are mainly used for meat and draught. These animals are very well adapted to hot and humid climates as well as marshy lands. With their large hooves they are able wade through the swamps and reach a variety of fodder sources (for review see Sastry, 1983; Mahadevan, 1992).

The Riverine breeds of the Indian subcontinent are mainly raised for milk production. Their milk yield is about 6 to 7 litres per day with an average content of fat, protein, and lactose of 7.5, 4.2, and 5%, respectively, which gives the buffalo milk a higher energy content than milk from dairy cattle (Rao & Nagarcenkar, 1977; Kay, 1994; Walstra *et al.*, 1999). Twelve of the 18 major Riverine breeds of buffaloes are kept primarily for milk production. The main milk breeds of India and Pakistan are the Murrah, Nili-Ravi, Surti, Mehsana, Nagpuri, and Jafarabadi. The river buffalo prefers, as its name reveals, to wallow in clear, running water (for review see Mahadevan, 1992)

Buffaloes are known to be good grazers, but they graze a wider range of plants than cattle (BSTID, 1981; Pathak, 1992). They utilize low-grade roughage more efficiently compared to cattle (Sebastian, Mudgal & Nair, 1970; Rao & Nagarcenkar, 1977). The unique ability of the buffaloes to survive under the most difficult conditions of nutrition and management has given it a competitive edge over other milk producers in the areas where buffaloes thrive. In addition, the buffalo has a long productive life. The normal healthy female buffalo could have as many as 9–10 lactations. (for review see Ganguli, 1981)

Although the economic importance of buffaloes has always been known, very little work has been done to exploit the genetic potentials of this animal by applying modern dairy farming practises for large-scale dairy production.

Limitations in buffalo milk production

Although the river buffalo is the main dairy animal in some countries, it is a primitive animal when compared to the developed dairy breeds among cattle like the Holstein-Friesian and Jersey. Many generations of selective breeding has produced cattle with almost predictable productive and reproductive traits. This has not been the case among the buffalo breeds as the majority of these animals are reared by land less and marginal farmers where they breed naturally (Chantalakhana & Falvey, 1999).

In general a dairy cow is considered to be efficient if the age at first calving is about 24 to 30 months. The calving interval should be about 12 to 13 months, lactation length of about 300 days, a 60 to 90-day dry period, with a production of between about 6000 to 7000 kg per lactation. As an example, in Sweden, the average milk produced per recorded dairy cow was 8794 kg, 4.14% fat and 3.40% protein or 8939 kg ECM (energy corrected milk, Sjaunja *et al.*, 1990). Age at first calving among the herds reported was around 29 months with calving intervals at about 13.2 months (Swedish Dairy Association, 2003). One could argue that the buffalo has its own species-specific productive and reproductive traits. In general buffaloes are usually around 40 to 60 months at first calving (Ganguli, 1981). However, there are indications that the productive traits can be improved. As an example, the Mediterranean breeds and the swamp buffaloes calved earlier than those of Indian subcontinent (Rao & Nagarcenkar, 1977). Average calving intervals for Indian and Pakistani buffaloes ranged from 15 to 18 months. The dry period has been reported to be 90 to 150 days for the Nili-Ravi breed of Pakistan while for the Murrah, it ranged from 60 to 200 days (Wahid, 1973). Average lactation length ranged from 252 to 270 days. As a result of these factors the productive life of a buffalo is only 39% of its total life when compared to 52% in developed dairy breeds (for reviews see Ganguli, 1981; Sastry, 1983).

In most of the buffalo milk-producing countries of Asia, it is observed that there are large seasonal variations in breeding and calving in buffaloes (for review see Ganguli, 1981). In India and Pakistan, 80% of the buffaloes calve during June and December causing a decline in milk production in the summer months. However, others have suggested this summer decline in milk production could be due to heat stress and shortage of greens. A dark body, lesser density of sweat glands and thick epidermis make it difficult for the buffaloes to thrive in extreme hot and dry conditions. Buffaloes have developed survival mechanisms to seek water for immersion in these conditions. When exposed to extreme hot or cold conditions, the buffaloes' milk production and reproductive efficiency are strongly affected (for review see Sastry, 1983). In addition to climatic influences, poor nutrition and management also affect breeding and production.

Contemporary innovations in buffalo production

Changes in breeding, feeding and management can bring notable improvements in productive and reproductive performance of buffaloes (for review see Sastry, 1983). An obvious, though neglected target has been finding out why the first calving occurs at such a high age. Close attention from the time of calving to the time heifers reach breedable body size could bring down the age at first calving by 6 to 9 months (Sastry & Tripathi, 1998). It has been shown that balanced feeding could bring the buffalo heifers into cyclicity when reaching 330 kg body weight. There are also cases where heifers have calved at 20-24 months (for review see Ganguli, 1981).

The calving intervals in buffaloes are influenced by the irregular and silent heat period as well as some reported hormonal irregularities (reproductive hormones) and seasonality. It has been reported that there is seasonal breeding in buffaloes due to a diminished sexual activity in the period between March and June (for review see Ganguli, 1981). Although buffaloes are thought to be seasonal breeders, it has also been reported that they can breed throughout the year if the reproduction management is good (Rao & Nagarcenkar,1977; Sastry & Tripathi, 1998). Thus, important management factors to consider in improving milk production are managing nutritional status around calving, pre- and post-partum hygiene, good milking management, balanced feeding, heat detection, and artificial insemination, managing thermal stress and improving housing (for reviews see Ganguli, 1981; Sastry, 1983). Among these management factors, milking has received the least attention according to the review article by Sastry & Tripathi (1998).

Milking management of buffaloes

Milking management can be regarded as one of the most important and crucial activities in the milk production chain. Much work has been done on the milking management of dairy cattle, sheep and goat, but comparatively little research data is available on the milking management of buffaloes (for review see Sastry & Tripathi, 1998). In general buffaloes are known to be difficult to milk. A number of researchers from different parts of the world have reported the problem of disturbed milk ejection and rapid termination of lactation in cases where the calves die or the usual milker is replaced (Aliev, 1969; Aliev, 1970; Cockrill, 1974a; Ragab, 1975; Pathak, 1992). In a survey conducted in India on the prevailing milking practises, it was reported that a majority of farmers experienced these problems with their buffaloes. In order to overcome the problems, 65% of the farms surveyed used concentrate feeding during pre-stimulation to improve milk let down, while 13% used injectable oxytocin to induce milk ejection (Varma & Sastry, 1994). Although not documented, it is well known from practise that in large buffalo herds, oxytocin injection is frequently used to achieve milk let down. The disadvantages with this have been reported recently by Bruckmaier (2003) who found that continuous oxytocin treatment could lead to addiction. To avoid unnecessary side-effects of these treatments, it is necessary to understand the factors that influence the efficient removal of milk in buffaloes, such as milk accumulation, storage of milk, and milk ejection.

Availability of the cisternal fraction, mode of milk ejection and emptying of the alveolar fraction, teat location, teat dimensions and teat sphincter resistance are some of the established factors known to influence the efficiency of machine milking in dairy animals. The modern dairy cow has fewer problems associated with these factors while machine milking because the animal has been also breed for milkablity (Bramley, 1992)

Teats and machine milking

Teat dimensions and stiffness due to being full with milk prior to milking, all have a bearing upon the complete removal of milk and stripping yields during machine milking. This has been reported for the machine-milked dairy species such as sheep, goat, and cattle (Mein *et al.*, 1973; Williams & Mein, 1982; Labussiere, 1988; Bruckmaier & Blum, 1998).

The rubber liners in the teat cups are the only parts of the milking machine that is in direct contact with the teats during machine milking. Ideally the internal diameter of the liner should match the external diameter of the teats of the herd being milked. The liners are designed to collapse cyclically or “pulsate” at least 50 to 60 times a minute based upon the specifications of the pulsator. The collapsing of the liner due to the pressure differential across the liner is known as the “relief phase” or “liner closed phase” of milking. In this position the liner is able to enclose the tip of the teat and exert pressure (compressive load) preventing accumulation of blood and lymph fluids at the teat tip (Williams & Mein, 1980; Mein, Williams & Thiel, 1987). If the teats are too long for a selected liner, the teats will penetrate deep into the liners causing pulsation failure which in turn can lead to incomplete milking and teat damage (Mein, Brown & Williams, 1983; Zeconni *et al.*, 1992).

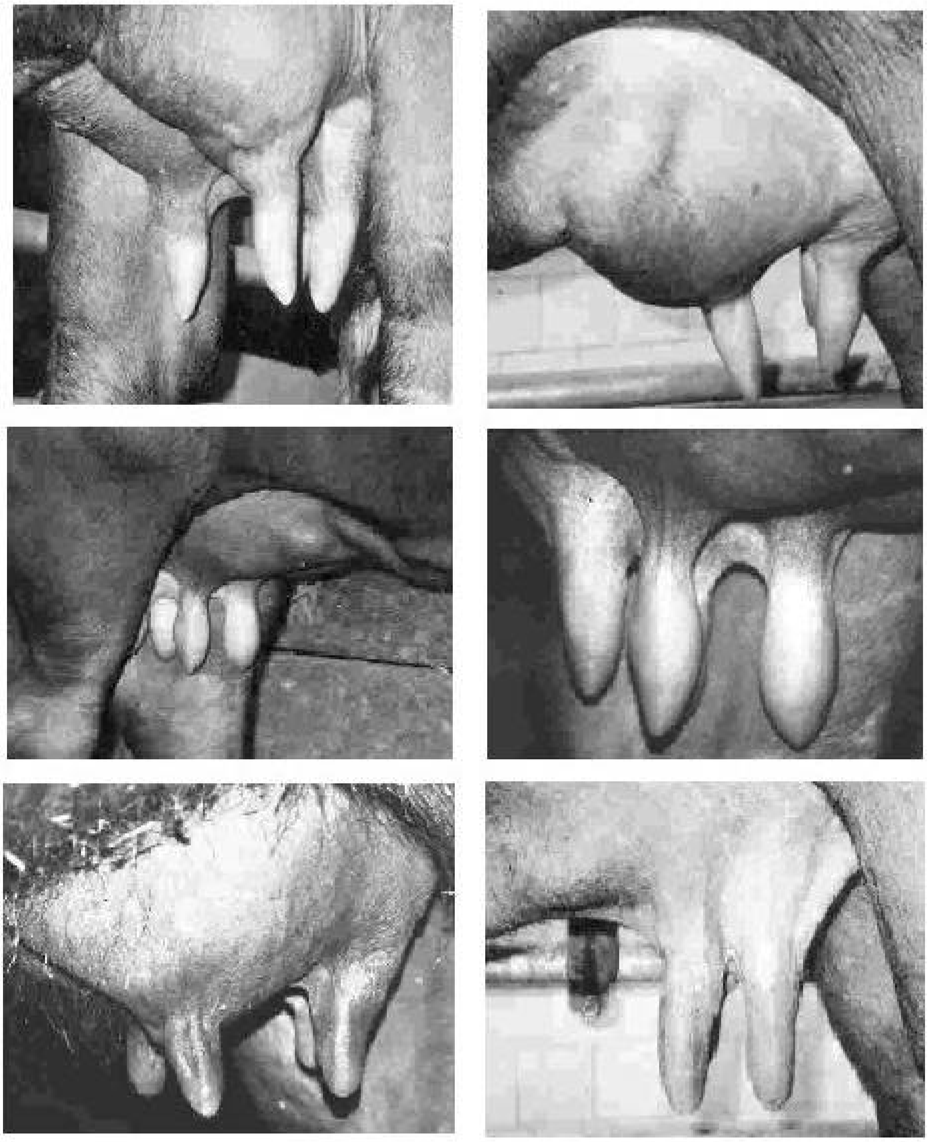

Figure 1. Udder shapes and teat sizes in Murrah buffaloes

The removal of milk from the teats during machine milking depends upon the efficiency of opening the teat orifice, the teat canal's effective diameter, and the intramammary pressure within the teat on account of being full with milk (Mein, 1992). When the milking unit is attached, the teat walls are stretched by the milking vacuum during the liner open phase, thus opening the teat canal by overcoming the biological closing forces exerted by the teat sphincter (Williams & Mein, 1982). When attaching the milking cluster, the teats move into the liner and there is a risk for it to "crawl" high up to the base of the teat due to the liner vacuum. This is known to happen toward the end of milking in cattle when the teats are empty. On the other hand, there is also the risk to fall or “slip” off due to the gravitational force. The frictional force of the liner prevents the cluster from crawling too high or falling off (Mein *et al.,* 1973). The rubber liners used for sheep, goats, and dairy cattle have been optimised through many years of research to correct for these problems. However buffaloes have longer teats and long teat canals compared to dairy cows, which is important to consider when machine milking them (Sastry, Bhagat & Bharadwaj, 1988; Saxena, 1973; Uppal *et al.*, 1994, Figure 1). In the absence of cisternal fraction of milk the teats of buffaloes would be empty (Aliev 1969), and thus while machine milking they could be similar to the teats of dairy cattle towards the end of milking. However, to my knowledge, teat dimensions before and after milk ejection and its influence on machine milkabliblity has not been studied thoroughly.

Table 1. *Different vacuum levels, pulsation rates and pulsation ratios used for milking buffaloes in different parts of the world.*

Year of publication	Country	Vacuum kPa	Pulsation rate (cycles/min)	Ratio of liner (open:closed)
Thomas & Anantkrishnan, 1949	India	46	50	-
Marathe & Whittlestone, 1957	India	68	40	50:50
Aliev, 1970	Azerbaijan	56	60	-
Alim, 1977	Egypt	51	60	50:50
Pazzona, 1989	Italy	45	60	60:40
Badran, 1992	Egypt	56	65	-
Lind *et al.,* 1997	India	56	70	65:35

Researchers from different parts of the world have reported relative difficulties regarding the milkablity of buffaloes where the animals have been described as “hard milkers” and other milk ejection-related problems have been highlighted (Thomas & Anantakrishnan, 1949; Marathe & Whittlestone, 1958; Aliev, 1969; Ragab, 1975; Gangwar, 1976; Alim, Barbari & Bardran 1977; Alim, 1982; Alim, 1983; Sastry *et al.,* 1988). Aliev (1969) also reported that a vacuum pressure of over 30 kPa was necessary to relax the teat sphincter in buffaloes. To overcome the problems when machine milking buffaloes, higher vacuum levels (10–15 kPa higher) than used for cattle have been used (Table 1). However, Thomas & Anantkrishnan (1949) in India and Pazzona (1989) in Italy used the levels similar to that for cattle.

Distribution and availability of milk in the mammary gland

Among the eutherians (95% of the mammalian species), the mammary gland has different anatomical structures, different locations, and numbers. However, in all species the mammary gland consists of secretory and ductal tissue (Mepham, 1987a). Among the prominent dairy species, like cattle, goats and sheep, as milk secretes it is transferred within the gland via the ducts into a large sinus (cistern) which drains out from a single orifice for each lobule (cluster of alveoli). As a consequence, a relatively large portion of the secreted milk is stored as the cisternal fraction of milk (within large ducts or galactophores and sinuses or cisterns) in these species. In other species including rats, rabbits, guinea-pigs and also humans, this sinus is either small with a single orifice or several ducts drain out of different orifices on a perforated nipple (Mepham, 1987a). Thus in these species, milk is almost entirely stored as the alveolar fraction in the alveolar lumen and small ducts.

Goats and sheep have two inguinal mammary glands, while cows and buffaloes have four. The cisternal area of the mammary gland in the dairy species is referred to as two separate cavities, the teat and gland cistern. Of the total milk secreted in 10 to 12 hours in cows, the cisternal fraction has been reported to be between 20 to 40% while in goats and sheep, the cisternal cavities are relatively larger than in cows (Peaker & Blatchford, 1988; Bruckmaier & Blum, 1992; Knight & Dewhurst, 1994). In studies on buffaloes of different breeds and stages of lactation, it has been observed that there was no cisternal fraction (Aliev, 1969).

The rate of milk secretion and the process of milk removal are both influenced by the size of the cistern (Wilde & Knight 1990; Wilde, Knight & Peaker, 1996; Stelwagen, 2001). In both sheep and cattle, it is established that animals with large cisterns are better producers of milk and are adapted to simple milking routines and longer milking intervals (Dewhurst & Knight, 1994; Knight & Dewhurst, 1994; Davis *et al.,* 1998; Labussiere, 1988). It is also reported that animals with small cisterns are more susceptible to the short-term autocrine inhibition of milk secretion in the mammary gland and are reported to respond better to more frequent milkings (Wilde *et al.,* 1996; Stelwagen *et al.,* 1998; Stelwagen, 2001)

The presence or absence of cisternal milk in the different dairy species is important when machine milking. In sheep and goats the large cisternal fraction milk is available for a comparatively longer time during machine milking and the lack of pre-stimulation therefore does not have a critical influence on milk removal (Peaker & Blatchford, 1988; Bruckmaier *et al.,* 1994a). In dairy cows machine milking without pre-stimulation can cause a temporary reduction in milk flow (bimodal milk flow) or a total interruption in milk flow after the cisternal fraction was extracted (Gorewit *et al.,* 1983; Lefcourt & Akers, 1984; Mayer *et al.,* 1984; Gorewit & Gassman, 1985; Philips, 1986). The consequence of this can be milking on "empty teats" until the alveolar milk is available. In sheep, goats, and cattle during machine milking emptying of the large cisternal fraction could stimulate the flow sensitive receptors situated in the teat canal and cisterns possibly resulting in a complete better ejection (Lefcourt, 1982; Vandeputte-Van Messom *et al.,* 1986; Hammon *et al.,* 1994; Roets, Burvenich & Hamann, 1995). To my knowledge, no studies have been done where consideration has been taken to the distribution of the cisternal size and fraction in buffaloes exposed to machine milking.

Various methods like corrosion casts, frozen sections, and ultrasound cross section of whole mammary glands have been used to measure cisternal area in cows, goats, and sheep (Bruckmaier *et al.,* 1992). Catheterisation of the teat was earlier used as a common method to measure the cisternal fraction while more recently oxytocin receptor blockade has been used successfully to prevent milk ejection prior to extraction of the cisternal fraction (Knight *et al.,* 1994; Wellnitz *et al.,* 1999). A combination of ultrasonography and oxytocin receptor blockade has been demonstrated to be an effective and non invasive method to determine the cisternal size and cisternal fraction in cattle (Ayadi *et al.,* 2003), and might therefore also be used in buffaloes.

Physiological implications on milk ejection and milk removal

The cisternal fraction of milk can be removed by simply overcoming the barrier of the teat sphincter. However, the alveolar milk fraction must be actively shifted into the cisternal cavities via the neuroendocrine reflex called milk ejection acting through the release of the pituitary hormone oxytocin (Crowley & Armstrong, 1992). Tactile stimulation like calf suckling, manual pre-stimulation, and the pulsating movement of the liner against the teat during machine milking, all stimulate the pressure-sensitive neural receptors located on the teat (Findlay *et al.*, 1966). Stimuli received by the neural receptors travel as nerve impulses via the central nervous system terminating at the supraoptic and paraventricular nuclie of the hypothalamus causing the release of the hormones oxytocin and prolactin in response (Cowie, Forsyth & Hart, 1980). As a consequence of increased blood levels of oxytocin, myoepithelial cells surrounding the secretory tissue in the udder contract and milk is expelled out into the cisternal area (Linzell, 1955; Soloff, 1980).

Milk ejection is a complex process involving a number of interactions between the mother and the young (Mepham, 1987b). However the efficiency of stimulation has a clear impact on the intensity in the pituitary release of oxytocin. For instance in rats it was observed that separating two or three pups from the nipples produced a proportional fall in the magnitude of oxytocin release (Lincoln & Wakerley, 1975).

Prolactin and cortisol are the other hormones released during milking (Gorewit *et al.*, 1992). Prolactin is known to influence milk synthesis and together with growth hormone it helps in maintaining milk secretion (Knight & Flint, 1995). The influence of cortisol on milk production is unclear. The stress of relocation and transportation cause an increase in cortisol levels (Varner *et al.*, 1983). However administering exogenous cortisol did not inhibit milk ejection (Mayer & Lefcourt, 1987).

In studies on sheep and cows in some breeds, varying responses have been reported to the different stimulation of milk ejection. Some breeds among the Zeebu only release oxytocin as a response to calf stimulation (Pegram *et al.*, 1991). Failure of oxytocin release was also reported in machine-milked ewes (Marnet *et al.*, 1999). It has been observed in dairy cows that different kinds of stimulation like the presence of the calf, suckling, manual pre-stimulation and feeding during milking increase the milking-related release of oxytocin concentration (Sagi, *et al.*, 1980; Akers & Lefcourt, 1982; Svennersten *et al.*, 1995; Lupoli *et al.*, 2001). In dairy cattle breeds like the Holstein and Jersey, many experiments have been performed over the last 40 to 50 years to study the effect of pre-stimulation. Some of the different types of tactile stimulus used during the pre-stimulation routines include washing the teats, wiping with a warm cloth, heated teat cups and other mechanical stimulation (Bilek & Zuda, 1959; Sagi, Gorewit & Zinn, 1980; Worstorff *et al.*, 1980; Karch, Worstoff & Prediger, 1989). The stimulation of the calf suckling evokes the strongest milk-ejection response while manual pre-stimulation is known to produce a better response than the stimulation provided by liner movement during machine milking (Akers & Lefcourt, 1982; Mayer *et al.*, 1984; Gorewit *et al.*, 1992, Bar–Peled *et al.*, 1995; Lupoli *et al.*, 2001). Manual pre-stimulation resulted in shorter milking time. Without manual-pre-stimulation, there was a temporary interruption in the milk flow curves when compared to machine milking (Gorewit & Gassman, 1885; Bruckmaier & Blum, 1996). In a study covering a complete lactation in dairy cows it was reported that with a strict pre-stimulation routine there was an increase the average milk yield and fat percentage in the lactation yield (Rasmussen *et al.,* 1990). The buffaloes' response to different pre-milking stimulations is not fully evaluated.

In dairy cows, it has been demonstrated that the stage of lactation due to the degree fullness of the udder influenced the time until milk ejection after the start of stimulation (Bruckmaier & Hilger, 2001). In practice, this aspect is especially important in milking animals in late lactation and during short milking intervals. It has been reported that buffaloes in later stages of lactation have a longer milk ejection time (Gangwar, 1976; Alim, 1982).

For optimal milk removal, it is reported that oxytocin concentrations need to remain increased over the basal levels during the entire milking (Bruckmaier, Schams & Blum, 1994b; Bruckmaier & Blum, 1996). It was also found that it was the timing of oxytocin release that was important and not the peak concentration for efficient removal of milk (Schams *et al.,* 1984). Insufficient amounts of oxytocin can cause incomplete milk ejection and the remaining milk in such cases can only be removed with another milk ejection or a supraphysiological dose of oxytocin (Bruckmaier & Blum, 1996; Bruckmaier, Pfeilsticker & Blum, 1996).

Inhibition of milk ejection in cows is reported at the peripheral level and at the central level. At the peripheral level it occurs by the stimulation of α-adrenergic receptors causing a contraction of the cisternal area and the duct system that restricts emptying of the udder. This inhibition can also occur by oxytocin receptor blockade (Bruckmaier, Mayer & Schams, 1991; Bruckmaier, Welnitz & Blum, 1997). Inhibition of milk ejection at the central level was observed when milk ejection and associated oxytocin release did not occur in relation to pre-stimulation, whereas normal milk ejection occurred after injection of a physiological dose of oxytocin (Bruckmaier, Shams & Blum 1992; Bruckmaier, Shams & Blum, 1993; Bruckmaier *et al.,* 1996).

Disturbed milk ejection occurred in unfamiliar surroundings in cows and did not cause an increase in oxytocin concentration over basal levels, and that case only the cisternal milk was possible to be removed (Bruckmaier *et al.,* 1993). Repeated occurrence of inhibition of milk ejection and high residual milk fractions could result in a reduction in the secretory activity in the mammary gland. This is reported to happen by way of an autocrine control mechanism due to the presence of a substance termed as the feedback inhibitor of lactation (FIL) resulting in mammary apoptosis (Wilde *et al.,* 1987: for review see Wilde & Peaker, 1990; Stefanon *et al.,* 2002)

Influence of management on maintenance behaviour and milk production in buffaloes

Integrating various aspects such as improved housing, nutrition, breeding and milking together are known to produce remarkable improvements in productive performance of buffaloes (Sastry & Tripathi, 1998). Better animal welfare will be reflected in the normal behavioural activities and milk production. In dairy cattle it is established that restricting normal feeding leads to behavioural abnormalities like tongue rolling (Redbo *et al.,* 1996). It has also been found that if there is competition for food or if feeding is restricted, then animals spend more time standing and waiting for food, or searching for food instead of resting (*e.g.* Olofsson, 2000). The daily rhythm of behavioural activities in cattle has been well documented and the influence of aspects such as social dominance, competition, and space availability have been studied (Olofsson & Wiktorsson, 2001; Friend, Polan & McGillard, 1977; Grant & Albright, 1995). In comparison, far fewer reports are available on the behavioural responses of buffaloes. In general the time budget of different behaviours in buffaloes is similar to those observed in cattle (Schultz *et al.*, 1977; Bud *et al.*, 1985, Odyuo *et al.*, 1994). However, these studies have not been made on buffaloes in a mechanised system.

The management routine of feeding during milking is known to increase milk flow and milk yield while it decreases machine on time and residual milk in cattle. It also seems to influence the general behaviour in dairy cows like total time spent standing, social interactions, and rumination (Samuelsson, Wahlberg & Svennersten, 1993; Svennersten *et al.*, 1995; Johansson *et al.*, 1999). As mentioned earlier, buffaloes seem to be very sensitive, and especially the milk ejection can be disturbed easily. Therefore it is necessary to know how small changes in the management routines will influence the milking process and behaviour during milking.

Influence of milking management and herd size on udder health

Mastitis, especially the subclinical form, is one of the most costly diseases in dairy production (DeGraves & Fetrow, 1993; Singh & Singh, 1993). It is not accompanied by any visible symptoms and may be undetected for a considerable time. The diagnosis must be based on factors that are altered in mastitic milk when compared to normal, referred to as inflammatory indicators. The background to mastitis is multifactorial and includes factors like individual anatomical and physiological characteristics of the animal, the general health and immunological defence, factors in the management and environment, and the interaction of these factors. Most factors predisposing for mastitis can be influenced by the management (DeGraves & Fetrow, 1993; Janzen, 1970; Woolford & Williamson, 1988; for review see Ekman 1998). The milking technique and the milking routines have a direct influence on udder health. Factors like good milking routines, clean and well-maintained milking equipment, and good teat end condition, are all reflected in the udder health status of a herd (for review see Carroll, 1977; Ekman 1998).

An inflammatory reaction is the body's response to an alien substance, *e.g.* a bacteria, bacterial metabolite or toxin, or tissue injury (Sandholm, 1995). The efficiency of the response in combating the cause is influenced by the immunological status of the animal. Management of dairy animals influences the occurrence of mastitis by way of infection, direct tissue damage and indirectly also by way of the immune defence through general management factors (for review see Carroll 1977). In mastitis, infection must be considered to be the most common cause of prominent inflammatory reactions. The risk for infection increases with increasing infectious pressure in the environment and with impaired function of the closing mechanism of the teat canal, which gives bacteria a good opportunity to invade the udder. The milking technique and routines are the main factors influencing the condition of the teat tissue. The degree of tissue trauma that may be caused by each milking might not be obvious but significant, because it is frequently repeated during the animal's entire lactation and may be one reason for general impaired herd udder health status. Milking by humans should as close as possible mimic the milk extraction by the suckling calf, which is ideal for the udder.

Both the herd size and the degree of mechanisation in the management may influence the occurrence of mastitis in cows if not special attention is paid to some factors. In big herds, it may be difficult to consider the animals' individual needs and more personnel are involved in the management, implying a potentially higher risk for health disturbances like mastitis (Carroll, 1977; Ekman, 1998). Mechanisation of the management, e.g. the milking, which *per se*, is an important tool to improve animal production can, if not controlled carefully, involve a potentially higher risk for negative influence on the animal health. A technical fault may give a systematic effect on the whole herd. Hence, a herdsman exercising diligent and informed management can influence and control the udder health status in the herd (Bramley & Neave, 1975; Bramley & Dodd, 1984; Goodger *et al.*, 1993). There is little information about how factors like the herd size, milking routines, and mechanisation of milking influence udder health in buffaloes. However it has been reported that buffaloes maintained in intensive systems in urban areas had a relatively higher incidence of mastitis compared to those maintained in backyard farming systems (Cockrill, 1974b).

The buffalo has traditionally been considered to be less susceptible to mastitis than cattle (Wanasinghe, 1985). Bansal *et al*., (1995) showed a lower occurrence of infectious mastitis in buffaloes than in cattle but Kalra & Dhanda, (1964) and Badran (1985) showed similar mastitis frequencies for the two species in the same geographical areas. There are some important differences in the properties of the buffalo udder compared to that of the bovine, which might influence the predisposition for infections and inflammation. The buffalo udder is more pendulous in comparison to cattle and has longer teats. These characteristics may contribute to higher risk of injury to the teats of buffaloes, predisposing for mastitis (Mohan, 1968). On the other hand, the buffalo has a long, narrow teat canal which can be expected to effectively prevent microorganisms from invading the udder and may be one reason for an eventual lower incidence of mastitis in buffaloes than in cattle (Krishnaswamy, Vedanayakam & Verma, 1965; Uppal *et al.*, 1994). The studies on the occurrence of mastitis in buffaloes have been based on different tests and parameters, sometimes with unclear criteria for defining mastitis and usually without clinical examination of the udders. The results are therefore difficult to interpret and hazardous to compare.

During inflammation, leukocytes in high numbers, mainly neutrophils, are recruited to the site of the reaction (Sandholm, 1995). In mastitis this causes an increased somatic cell count, SCC, in the milk. In the bovine, SCC is the most commonly used inflammatory indicator to diagnose subclinical mastitis. The relation between the SCC and proportion of neutrophils in bovine mastitic milk has been studied for several decades and the proportion of neutrophils is considered to be a more sensitive measure of inflammation in the udder than the total SCC (Östensson, Hageltorn & Åström, 1988; Miller, Paape & Fulton, 1991; Pillai *et al.*, 2001). Somatic cell count has been used in mastitis diagnosis to a limited extent in buffaloes (Singh *et al*., 1982; Dhakal, 1994, Singh & Ludri, 2001). Dhakal (1992) and Silva & Silva (1994) additionally studied the relation between the SCC and the proportion of neutrophils. So far a reliable threshold value for a normal SCC in buffalo milk has not been possible to set but according to these studies, it seems probable that a SCC > 200×10^3/ml is indicative of mastitis. The California Mastitis Test (CMT), which was developed as a cow side test for bovine milk to estimate the cell content, has been used in buffalo milk (Singh *et al.*, 1982;

Bhindwale, Supekar & Shukla, 1992; Dhakal, 1994). It has been assumed that the interpretation of the test results should be the same as it is in bovine milk, without any proper evaluation of the test when used in buffalo milk.

Objectives

The general aim of this study was to investigate the influence of teat dimensions and partitioning of milk in the udder on milk removal during machine milking. The aim was also to understand the physiological and behavioural responses of buffaloes to pre-milking routines on milk flow characteristics and the impact of milking routines on udder health. The specific objectives of the current study are as stated below:

- To estimate the teat length and teat girth (thickness) and the cisternal area size before and after milk ejection and also to estimate the teat canal length in buffaloes.
- To estimate the cavity area within the mammary gland of buffaloes and determine the partitioning of the cavity area in the teat and gland region of the udder. Similarly to estimate the fraction of milk stored in the cavity areas of the udder in comparison to the total volume of milk secreted in an interval of 10 to 11 hours.
- To investigate the effects of different pre-milking routines on the commencement of milk flow, average and peak flow, milking time and emptying of the udder compared to induced milk ejection at physiological levels.
- To investigate the milking-related release of oxytocin, prolactin, and cortisol in response to different pre-milking routines and the relation to milking characteristics and stripping yield.
- To study the maintenance behaviour and behaviour during milking in a mechanised production system and investigate the influence of small changes in management upon maintenance behaviour, behaviour during milking, and milking characteristics.
- To survey the impact of herd size and milking management systems on udder health.

Materials and methods

The study was performed during 2000–2002 in the Sangli district in the southwest of India. Four of the experiments (paper I, II, III and IV) included in this thesis were conducted on an experimental farm, B.G. Chitale Dairy Pvt., Ltd. and one study (paper V) was done as a field survey on farms in the vicinity of the research farm. Chitale Dairy Farm is a bull mother farm and a progeny-testing centre approved by the Indian Council of Agricultural Research.

Animals and management

Studies done at the research farm

The herd on the farm consisted of 150 high-producing Murrah buffaloes and their followers. Average lactation production was around 2500 kg although there were a few animals producing more than 3000 kg. The average fat content in milk among these animals was 7.5%, average age at first calving was around 38 months, average inter-calving period was 410 days, and average lactation length was around 300 days.

The lactating buffaloes were kept in a loose housing system, which was divided in four groups, each housing 24 milking animals. The young stock and the dry animals were kept in an adjoining barn. The barns were equipped with systems for mechanised milking, concentrate feeding, water and manure handling. Each resting-place had a micro-sprinkler fitted two meters above that was turned on once during the afternoon. Buffaloes also received a shower before being milked two times a day at 07.00 and 17.00 hours (h) in a tandem parlour.

The buffaloes were fed roughages on a fresh weight basis. The roughage consisted of a mix of feeds available in different seasons (paper IV). A pre-calculated concentrate ration based on the milk composition, milk yield, body weight, and pregnancy status was fed through the concentrate feeding system. All animals were fed concentrate during milking (for more details see paper IV).

In the first two experiments (paper I and II) 24 buffaloes in three different stages of lactation and two parity classes were selected to estimate the cisternal area size and the cisternal fraction. Measurement of the cisternal area size was done in a treatment clinic adjoining the barn where they were housed (paper I). Milk ejection and milk flow characteristics in response to different treatments (paper II) were determined in the milking parlour. In the third experiment (paper III) six buffaloes in the same lactation stage and from a similar parity class were selected. All buffaloes in this experiment were provided with a semi-permanent catheter in the jugular vein two days prior to the start of the experiment. After catheterisation they were housed in the treatment clinic, but they were machine milked with different treatments in the milking parlour (for details see paper III). In the behaviour study, 14 buffaloes from three different lactation stages were selected. All observations were made within the premises of the barn and the milking parlour (for details see paper IV).

Field survey

The survey (paper V) included 24 selected farms that were located at a radius of about 50 kilometres from the B. G. Chitale Dairy farm. Three different milking management systems and two herd sizes were represented in the study. Seven or eight animals were randomly selected at each farm for the study. Most of the buffaloes in the survey were of the Murrah breed except in the case of two farms where also buffaloes of the Phandarpuri breed were included (for more details see paper V).

Experimental design

Studies done at the research farm

Experiment one and two were performed simultaneously in two phases. The 24 buffaloes were grouped in three groups depending on the lactation stage (early, mid, and late lactation).

In the first phase, measurements were recorded for cisternal areas, cisternal volume of milk, time until milk ejection into the cisternal cavity in response to exogenous oxytocin, and commencement of milk flow in response to oxytocin injection (Paper I and II). All registrations were performed once in the afternoon at 16.00—17.30 h *i.e.* 10–11 h after the preceding milking. To avoid carry-over effects from the previous treatment, the animals were studied in experimental groups of six each in random sequence. Two animals from each stage of lactation had to undergo ultrasound measurements; cisternal volumes were measured in two more animals; and, commencement of milk flow in response to oxytocin injection was recorded in another two animals. Each treatment day was followed by a rest period of three milkings after which the next treatment was performed in a random sequence. In six days, all animals in one group received all treatments. This schedule was followed for all groups.

The second phase was initiated for each batch of animals after a rest period of three milkings. Four pre-milking treatments were administered: no pre-stimulation; a one minute pre-stimulation; a three min pre-stimulation; and a Duovac™ milking (DeLaval, AB Sweden). Each animal received the same treatment during four milkings prior to the registration milking done on last evening milking of the treatment (paper II).

In the third experiment (paper III), blood samples were taken for analysis of the hormones oxytocin, prolactin and cortisol. After catheterisation of the jugular vein, the buffaloes during four milkings were accustomed to the new environment before the experiment started. The experiment was divided into three periods each including three different treatments, which were administered randomly. The three milking routines were milking with pre-stimulation and in-parlour feeding (MF), milking with pre-stimulation but without in-parlour feeding (M) and milking without pre-stimulation or feeding (O). In each period, all animals were allowed one morning and one evening milking for conditioning to the treatment followed by registration milkings the following morning.

Experiment four (paper IV) lasted for 20 days and consisted of two treatment. In period one (10 days) the normal existing feeding and milking regime of feeding three times a day was used (T-I). A staggered 24-h study of the maintenance behaviour in three days was done in the beginning and end of this period. During the four days between the beginning and end of the period, the milking characteristics and behaviour during morning and evening milkings were recorded. In the second period, a treatment with access to increased amount of roughage through two extra feedings along with a 50% reduction in the in-parlour feeding was compared to period one (T-II). In T-II, the animals were conditioned to the new treatment for five days, then observed during a continuous 24-h maintenance behaviour study, and during the last four days, the milking characteristics and behaviour during milking were recorded. This phase ended with another continuous 24-h study. However, the observations on milking on day four of the behaviour study overlapped with the 24-h study.

Field survey

In the field survey (paper V), the farms were categorised based on their herd size as large when having a herd size above 30 buffaloes and small when having a herd size between 10–20 buffaloes. Within each herd size three different milking management systems were practised: calf stimulation and hand milking (CH), manual stimulation and hand milking (MH) and manual pre-stimulation and machine milking (MM). Sample collection was done during the morning milkings.

Equipment and registration

Cisternal area and cisternal fraction

Cisternal cavity area size (teat and gland) and length of the teat canal were measured using B-mode ultrasonography (paper I). The udder was immersed in a water bucket as described by Bruckmaier & Blum (1992). A 6 MHz linear array rectal probe (Philips Medical Systems, India, Ltd.) was used. Cisternal size (area) and teat canal length were measured from the ultrasound pictures by means of a digitizing tablet combined with a personal computer program (Sigma-Scan®, 1988). Time to milk ejection into the cisternal cavities was recorded after injecting 2 i.u. oxytocin intravenously (Paratoxin® vet 10 i.u./ml, Pharmacia & Upjohn Animal Health AB, Helsingborg, Sweden).

The cisternal milk fraction in paper I was determined by preventing endogenous oxytocin release with an intravenous injection of oxytocin receptor blocking agent (Atosiban 10 mg/animal; Ferring Research Institute AB, Malmö, Sweden, Wellnitz *et al.,* 1999). A supra-physiological intravenous injection of 10 i.u. oxytocin was administered subsequently to abolish the oxytocin receptor blockade and obtain alveolar milk. Cisternal and alveolar fractions were recorded with a mobile milk flow recorder (Lactocorder®, WMB, Balgach, Switzerland) adapted to low milk-flow rates. A bucket-milking machine (DeLaval AB, Tumba, Sweden) was used for milking when the cisternal and alveolar fractions were recorded (paper I) and where milking characteristics in response to oxytocin injection were recorded (paper II).

Milking characteristics

All registrations on milking characteristics (paper II, III and IV) were done in a 2 × 5 low-line tandem parlour with an automatic in-parlour feeding system (DeLaval AB, Tumba, Sweden; operational vacuum 50 kPa, pulsation 70 cycles /min at a ratio of 65:35). The parlour was provided with Duovac milking units (for more details see paper II). Time until commencement of milk flow (milk ejection), average and peak milk flow, total milk yield and total machine on time were recorded for all treatments in experiment two using the Lactocorder (paper II). All animals were hand stripped and the stripping yield was measured with the Lactocorder by pouring it into one of the teat cups of the milking units. In experiment three and four (paper III and IV) milking characteristics were measured with the Flowmasterpro™ milk flow meter (ICAR approved, DeLaval AB, Tumba, Sweden) of the ALPRO™ milking system (DeLaval AB, Sweden). Milk ejection was recorded visually as the first time commencement of milk flow occurred without further interruption for at least 60 s into the transparent claw of the milking unit. A stopwatch was used to record time from the application of the first teat cup until milk ejection. Machine-on time was calculated as the time from cluster application until manual removal of the cluster when milk flow ceased. In experiment three the stripping yield was measured using a measuring cylinder with 1000 ml (approx. 1033 kg) capacity. In experiment four, stripping yield was measured in the same way as described for experiment one and two but using the Flowmasterpro milk meter.

Milking-related hormonal responses

In the third experiment (paper III) 10-ml blood samples were collected in ice-chilled K3-EDTA tubes (Sarstedt, Landskrona, Sweden) provided with Trasylol® (400 i.u./ml; Bayer Sverige AB, Gothenburg, Sweden). The samples were centrifuged at 1500 g for 15 min and the plasma was stored at –20°C until assayed. Blood samples were taken at 15 and 5 min before milking in the tie stalls. Thereafter, the animals were escorted into the milking parlour and blood sampling was continued at –1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +12, +14, +16 min around milking during the different treatments. At time 0 the milking cluster was attached to the udder.

Sample collection for milk analysis

Milk samples (10 ml) were taken from the composite milk and stripping yield (paper III). The milk samples were stored at 4°C and analysed the same day.

Behaviour

In experiment four, maintenance behaviour and behaviour during milking were recorded in separate ethograms. All behaviours to be studied were pre-defined based on previous studies (Schultz, *et al.*, 1977; Thind & Gill, 1986; Odyuo, *et al.*, 1994). Prior to the start of the study, the ethogram was tested and modified. Details of the definitions of the general behaviours and behaviour during milking are presented in paper IV.

Sample and data collection in the field survey

In experiment five (paper V), for each animal, data on lactation number, lactation month and milk yield at the morning milking were given by the farmer. The milk was inspected before milking and 10 ml of quarter milk were collected in clean tubes to be used for CMT and total and differential cell counting done later in the laboratory. After milking, a 2-5 ml quarter milk sample was collected aseptically as per National Mastitis Council (NMC, 1999) recommendations in sterile disposable tubes with screw cap and used for bacteriological analysis, and the udders were clinically examined. Bacteriological examination could not be performed on fresh samples because of the long distance to the bacteriological laboratory. The samples were stored at –20 °C within 6 h after sampling and kept there until they were transported to the laboratory.

Analysis

Blood samples were analysed for contents of oxytocin and prolactin by radioimmunoassay as described elsewhere for oxytocin (Schams, 1983) and prolactin (Bruckmaier *et al.*, 1992). Cortisol was analysed with an enzyme immunoassay as described by Sauerwein, Duersch & Meyer (1991) (paper III).

Milk samples from the main milk and stripping milk were analysed for fat, protein, and lactose using the mid infrared spectroscopy technique (Milkoscan 93, Foss Electric, Hillerød, Denmark) (Paper III).

CMT (Schalm & Noorlander, 1957; Schalm, Carroll & Jain, 1971) was performed and smears for cell counting were prepared at the laboratory within 6 h after the milk collection (paper V). Microscopic total and differential cell counting was performed according to Prescott and Breed (1910) modified by Åström (1972). The bacteriological examination was performed at a routine bacteriological laboratory, the Poultry Diagnostic and Research Centre (PDRC), Pune, India (Venkateshwara Hatcheries Ltd.), according to the recommended tests and standard procedures (Honkanen-Buzalski & Seuna, 1995; Laboratory handbook on bovine mastitis, 1999).

Statistical analysis

Statistical methods and models used are described in detail in each paper. When the general linear model was used for the analysis of variances, the statistical software used was Minitab™ (2000). When the MIXED model procedure was used for the analysis of variance the statistical software used was SAS statistical package (SAS, 1997). However, procedure GLM of SAS was also used in the analysis of variance (paper IV). The least square of means was used to detect differences between means. Pearsons correlation were used in papers I and II while Spearman rank correlation was used in paper V. In paper V, the generalized linear mixed models using the SAS macro GLIMMIX were used for categorical data (Littell *et al.*, 1996). The same model used for ANOVA with fixed and random factors was used for analysing the categorical data from the bacteriology results as well as the CMT results and proportion of neutrophils >30%.

Results

Cisternal area, cisternal fraction and teat dimensions

In this study, it was observed that in Murrah buffaloes the fore and hind teat length teats ranged from 5 to 14 cm and 8 to 16 cm, respectively. The teat girth (thickness) ranged from

7 to 14 cm and 8 to 16 cm, respectively. Upon milk ejection there was more than a 10% increase in the teat length and teat girth (paper I, Figure 2).

Buffaloes had a mean total cisternal area (teat and gland) of around 22 cm^2 for a single quarter which is less than half of that which is seen in cows (40–45 cm^2 and relatively higher in sheep and goat) and the cisterns were significantly larger in early compared to late lactation. The cavity area in the teat and gland regions were similar. The teat canal length in buffaloes was around 3 cm and was much longer compared to the 0.5 to 1.5 length reported in cows (paper I).

Cisternal milk fraction was lower than reports in cattle and goat (20 to 40%) and was only 5% of the total milk. There was a close correlation between the cisternal area measured using the ultrasound technique and the cisternal milk yield (paper I). During milk ejection, teat length and circumference, and the cisternal area in the ultrasound cross sections increased significantly (Paper I).

Milking characteristics

When milk ejection was induced by exogenous oxytocin at a physiological level, the time until milk ejection was similar to reports in cows *i.e.* 20 to 30 s and it was negatively correlated with the milk yield. With the pre-milking routines, one and three minute manual pre-stimulation and Duovac milking, commencement of milk flow was earlier compared to the treatment no stimulation (Paper II). Average flow was lower and machine-on time was longer when buffaloes were milked without pre-stimulation compared to manual pre-stimulation. As a fraction of total yield, machine yield was significantly lower while stripping yield was significantly higher in the treatment without pre-stimulation compared to the treatment one-minute pre-stimulation (P <0.05, paper II)

It was also observed that when buffaloes were milked without any pre-stimulation treatment, the stripping yield was significantly higher and fat content in the stripping yield lower compared to routines when the animals were manually pre-stimulated and fed concentrates (P <0.05, paper III).

Furthermore, when the amount of concentrate fed during milking was reduced by 50%, the commencement of milk flow and time to reach 500g of milk was significantly influenced (P <0.05). The milk flow was reduced and the time to reach 500 g was increased (paper IV). It was observed that stage of lactation had an influence on the milking characteristics. The average milk flow rate was higher in early lactation stages (P <0.05, paper II and IV).

Milking-related hormonal responses

A milking-related release of oxytocin, prolactin, and cortisol was observed when the buffaloes were milked with the routine manual pre-stimulation and feeding during milking (MF) (paper III). The routines of only manual pre-stimulation (M) and milking without pre-stimulation (O) gave a reduced milking-related response of oxytocin. As a consequence to the hormonal response, milk ejection occurred significantly earlier in the combined treatment of feeding and manual prestimulation (MF) than M and O (P <0.05). A small increase of >3–5 ng/l in oxytocin concentration over the basal levels was sufficient to induce milk ejection. There was a positive correlation between time untill increase in oxytocin

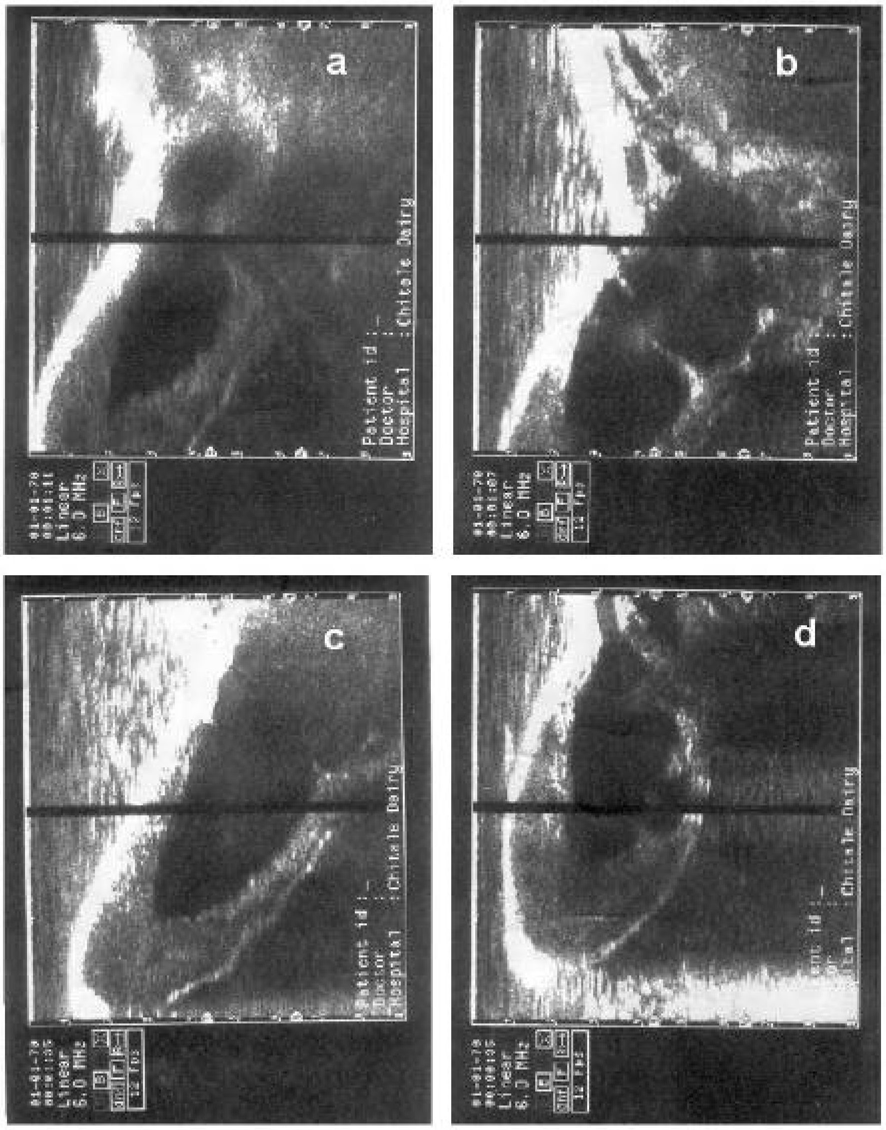

Figure 2. Ultrasound cross-section of the right half of a buffalo udder, a. right fore udder cistern, b. right hind udder cistern, c. right fore teat cistern and d. right hind teat cistern.

concentration and time until milk ejection. There was also a positive correlation between total time the oxytocin concentration remained elevated over threshold levels and machine yield. In treatment O, milk ejection was not observed during machine milking but oxytocin increased during hand stripping. In treatment M, although oxytocin concentrations remained low during pre-stimulation, it increased more than two–fold during subsequent machine milking and hand stripping. In treatment MF, there was more than a two-fold increase in plasma oxytocin concentrations during pre-stimulation and it continued to increase up to a peak and remained high throughout machine milking. Milking-related cortisol release was evident only in treatment MF. In treatment O and M, prolactin concentration increased prior to the increase in oxytocin where as prolactin and cortisol release followed the release of oxytocin in MF.

Maintenance behaviour and behaviour during milking

During 24-h observations, buffaloes spent on an average 23, 39 and 33% of their time eating, resting, and standing, respectively. Significant diurnal differences were seen in all behaviours like eating, standing, resting, except walking and lying idle. When the buffaloes were exposed to a treatment with increased availability of roughage (T-II) compared to routine feeding (T-I) some effects were seen on their behaviour. The rumination behaviour was significantly longer while standing during the night in T-I while in T-II this difference was not seen. In T-II they were lying for a significantly longer time whereas the total sleeping behaviour was significantly longer in T-II than T-I (P <0.05). Buffaloes in late lactation spent significantly more time walking and eating roughage and resting while time spent standing was lower than the other stages (P <0.05).

In T-II as a consequence of reducing the amount of concentrate fed during milking a significantly higher oral behaviour in the parlour was also seen compared to T-I (P <0.05) along with the above described effects on milking characteristics.

Influence of milking management and herd size on udder health

In the sampled population, the prevalence of mastitis based on increased value of the different inflammatory indicators was on an *animal basis*: CMT 38%; SCC 32%; and proportion of neutrophils 21%, respectively (paper V). On a *quarter basis*, the corresponding figures for prevalence of mastitis were: CMT 15%; SCC 14%; and proportion of neutrophils 8%, respectively. The prevalence of infectious mastitis was 33% on an animal basis and 12% on a quarter basis. Of the total quarters examined, 97.5% did not show any clinical sign of mastitis

The milking management system significantly influenced the frequency of quarters with increased SCC and infection while herd size had a significant influence only on the frequency of quarters with increased SCC. However, herd size also influenced the frequency of infection significantly but only by interaction with milking management system.

CH herds had the lowest prevalence of mastitis as measured by each inflammatory indicator and in small CH herds also as measured by infection. Large herds with MM had the highest prevalence of mastitis. In small herds with MM the prevalence was markedly lower and based on SCC and proportion of neutrophils even lower than the prevalence in the small herds with MH. The udder health was generally better among the small herds. However, in herds with MH, the prevalence of infection was higher in small than in large herds. Among infected quarters, environmental bacteria were predominant, with large MM herds having the highest prevalence.

The cell types found in the milk were monocyte-macrophages, neutrophils, lymphocytes and epithelial cells. In approximately half of the milk samples, structures with a characteristic appearance, looking like fragments of cells, were found, sometimes in high numbers. On average, milk with low SCC contained predominantly monocyte-macrophages while milk with high SCC contained predominantly neutrophils. The average proportion of neutrophils was increasing with increasing SCC. The mean SCC for each CMT score showed a good agreement with the values given in bovine milk (Schalm *et al.*, 1971). However, the correlation between CMT, SCC, and increased proportion of neutrophils was weak. The correlation between these factors was higher among the bacteriologically positive samples with high SCC.

Discussion

In this study several findings of practical importance to efficient machine milking of buffaloes were observed. It was evident that buffaloes have smaller cisternal area sizes and cisternal fraction of milk (paper I) than in the other dairy breeds among cattle, sheep, and goats (Peaker & Blatchford, 1988; Bruckmaier & Blum 1992; Knight & Dewhurst 1994; Caja *et al.*, 1999). Buffalo teats were flaccid and empty due to the small cisternal fraction of milk prior to milk ejection, while during milk ejection, there was a remarkable increase in teat dimensions (paper I). Also due to the small cisternal fraction of milk, commencement of milk flow during machine milking took longer without pre-stimulation (paper II) than in the other-mentioned dairy breeds (Bruckmaier & Blum, 1996). However, with the combination of manual pre-stimulation and feeding, there was an early release of oxytocin (paper III). Manual pre-stimulation and feeding during milking produced a quicker and more-pronounced release of oxytocin, prolactin, and cortisol, which is similar to reports in the other mentioned dairy breeds (Svennersten *et al.*, 1995; Johansson, Svennersten-Sjaunja & Uvnäs-Moberg, 2000). Changes in the milking routines like not feeding during pre-stimualtion or reduced concentrate feeding during milking had an immediate influence on oxytocin release, milk ejection, and complete removal of milk and this was reflected in the percent fat in strip milk (paper III and IV). The daily pre-milking routines, milking methods, and the management could have an impact on the udder health status of buffaloes (paper V). This was evident from the lower prevalence of mastitis and udder inflammation in the small buffalo farms, where the buffaloes were stimulated by calf suckling and hand milked compared to large farms where the buffaloes were manually pre-stimulated and hand milked or machine milked. In this study, for the first time we measured the cisternal area size and cisternal fraction of milk in buffaloes, furthermore, the milking related release of the hormones oxytocin, prolactin and cortisol in response to different stimulations was studied for the first time.

Milkablity of buffaloes

It is well established that the dairy cow's teat canal length, the effective diameter and the strength of the sphincter influences the milk-flow rate and udder health (Nickerson, 1992). In this study, we observed that buffaloes have much longer teat canals than is reported in dairy cows (paper I). In a recent comparative anatomical study between cattle and Egyptian buffaloes in slaughtered animals, it was reported that teat canals were 30 to 40% longer in buffaloes than in the Holstein cows (El-Ghousien *et al.*, 2002). It has also been reported that the epithelial thickness of the buffalo streak canal was about 10% greater and the thickness of the sphincter was 13 % greater than in cross-bred Holstien cows (Singh & Roy, 2003; Uppal *et al.*, 1994)

While machine milking buffaloes, the milk has to travel through a longer and narrower teat canal compared to cattle, and an apparently stronger muscle resistance has to be overcome to draw the milk out from the teats. This was evident from the earlier study by Aliev (1969) where it was shown that vacuum levels over 30kPa was necessary to open the buffalo teat canal and extract milk even after milk ejection. In cows it has been seen that less than 20 kPa is enough to open the teat canal (personal communication R. M. Bruckmaier; Bruckmaier, Weinfurtner & Weiss, 2004). This might be the reason why much higher vacuum levels have been used for milking buffaloes than those used for cattle (Thomas & Anantakrishnan, 1949; Marathe & Whittlestone, 1958; Aliev, 1969; Alim, 1982). However, milk can flow through the open teat canal only if the intramammary pressure due to milk accumulation in the cistern is higher than that of the milking vacuum (Williams & Mein, 1986). Aliev (1969) reported that the initial intramammary pressure in buffaloes was close to atmospheric pressure and that it took about two minutes to build up to an individual maximum after milk ejection. In cows it is reported that there is an intramammary pressure of around 2–3 kPa due to the hydrostatic pressure exerted by the cisternal milk which rises up to an individual maximum on milk ejection (Tucker, Reece & Mather, 1961; Hamman & Dodd, 1992; Bruckmaier & Blum, 1996).

In this study, it was evident that following milk ejection there was a remarkable increase in teat length, teat circumference and in the teat and udder cisternal area compared to that before milk ejection (paper I). This increase could be on account of the increased intrammary pressure after milk ejection. Thus prior to milk ejection, the teats have lesser tone as they are empty and limp. Therefore, it is necessary to investigate further whether synchronising the cluster attachment with the timing of milk ejection could make it possible to machine milk at slightly lower vacuum levels. Thomas & Anantkrishnan (1949) in India and Pazzona (1989) in Italy have reported that buffaloes can be milked at vacuum levels similar to that used for cattle. In Italy, however, buffaloes have been milked for over 30 to 40 years at the same vacuum levels and pulsation ratio used for cattle. It is not clear whether the buffaloes in Italy have been bred for better milkablity. To my knowledge other than Pazzona (1989) there are very few studies done on the machine milkablity of Italian buffaloes.

It is known that higher vacuum levels and vacuum fluctuations at the teat will influence the teat opening damage through the prolapse of the teat canal and injury, (Bramley, 1992). Higher vacuum and vacuum fluctuations are also known to cause the "impact" or "shot" effect whereby droplets of milk or other particles penetrate the teat canal. This impact could increase the risk for udder infections (Wilson, 1958; Jackson, 1971; Isaksson & Åström, 1988). Badran (1992) reported that in Egyptian buffaloes there was a significant increase in the SCC and leukocyte count beyond 56 kPa and pulsation rate of 65 cycles/min. The vacuum levels on the surveyed farms in this study when milking machines were installed was: vacuum 55 kPa, pulsation 70 cycles/min at a ratio of 65:35. In the present study, the average SCC in machine milked herds were not significantly different compared to the herds of the same size that were calf stimulated or hand milked (paper V). Among large herds, the prevalence of mastitis, based on each inflammatory marker was higher in the machine-milked herds when compared to the calf-stimulated and hand-milked herds. However, in the small machine milked herds the prevalence was markedly lower and based on SCC and percentage neutrophils it was even lower than for hand milked herds. Thus machine milking *per se* appears not to be the main reason for impaired udder health conditions. This is further discussed in a later chapter. In the light of the very low cisternal volume of milk and the use of relatively higher levels of vacuum in milking the Indian buffaloes, it is necessary to minimise the effect of vacuum in the period when there is no milk flow from the teats. An example of machine milking of buffaloes is given in Figure 3.

Figure 3. Machine milking of Murrah buffaloes with a bucket milking machine and pipe line milking system in India.

The other problems in machine milking of buffaloes are the cluster "crawl", cluster "slip" at the beginning and end of milking and stripping volume of milk (Marathe & Whittlestone, 1957; Pazzona, 1989). Owing to the small cisternal size and empty teats, attaching the milking cluster prior to milk ejection will cause the teats to be sucked too deep into the liner. This could causes the closure of the udder teat passage and results in incomplete milking. This has been reported in cows to happen towards the end of milking (Schams *et al.,* 1984; Bruckmaier & Hilger, 2001). In such a case the vacuum in the teat approaches the vacuum in the long milk tube and could lead to irritation of the tissue and tissue damage and could result in inhibition of milk ejection as reported in cows (Bruckmaier & Blum, 1998). It is also known that over-milking coupled with fluctuating vacuum and high vacuum levels intensify damage to the teat orifice of the mucous membrane lining of the teat cistern (Peterson, 1964). In the current study it was observed that milk ejection and related oxytocin release was significantly delayed without manual pre-stimulation. Although not verified this could also be as a consequence of inhibition of milk ejection due to irritation of udder tissue when milking at high vacuum levels on empty teats (paper II, III). Cluster slips have also been associated with mastitis in cows (O'Callaghan *et al.,* 1976).

In experiment one (paper I) as well as in previous studies, it has been reported that in buffalo teats are on an average much longer than in cows (Sastry *et al.,* 1988; Saxena, 1973). Machine milking of buffaloes when the teats are empty could lead to the teats entering deeply into the barrel of the liner and even more so if the liners are over sized. In cow it is reported that pulsation failure will occur if teat penetration is too high (Mein *et al.,* 1983; Rønningen & Reitan, 1990). Pulsation failure causes an increase in the teat end thickness by accumulation of tissue fluids. In cattle, an increase in teat end thickness of more that 5%

after milking than before milking increases the risk of colonisation by environmental pathogens (Zecconi *et al.*, 1992, 1996). Isaksson & Lind, (1992) reported that an increase in the teat tip thickness while machine milking depends on whether or not there is a milk flow from the teat. It is also known that the lack of pulsation in pulsation-less milking machines increased incidence of mastitis and decreased cow discomfort (Bramley, Griffin & Grindal, 1978; Woolford & Phillips, 1978). Thus, further studies on the teat reactions in buffaloes to different vacuum levels and pulsation ratios and the impact on inflammation and infection is necessary to improve machine milking in buffaloes. In this study, the prevalence of mastitis with environmental bacteria was found to be highest in large machine-milked herds (paper V). The factors that usually contribute to udder health problems related machine milking include poor hygiene, improper use of machines by over milking, improper cluster removal, worn out liners, and unserviced machines (Bramley, 1992).

The studies presented in this thesis clearly point to the need to verify and establish the machine milking parameters and develop ISO standards for machine milking of buffaloes. Optimising of the liner dimensions for correct teat positioning within the liner and optimising of the cluster weight for minimum slips are critical steps for efficient milking of buffaloes. This is crucial for improving milking efficiency and welfare of buffaloes.

Cisternal area size and partitioning of milk in the buffalo udder

From this study, it is evident that buffaloes store only a small fraction of milk in the cisternal cavities after a milking interval of 10 to 11 h (paper I). In cows it has been demonstrated recently that an 8-h interval is optimum for measuring the cisternal size and cisternal fraction of milk (Ayadi *et al.*, 2003). In the only earlier study on the partitioning of milk in buffaloes, Aliev (1969) studied different breeds, age, and stages of lactation and reported that there was almost no cisternal fraction found in buffaloes. This indicates that most of the milk synthesised between milkings is stored in the alveolar lumen and the small ducts and there is very little drainage of milk into the cisternal cavities. The completeness of emptying the udder during milking of buffaloes is entirely dependent upon timing of oxytocin release in response to stimulation and its sustained release through the entire milking (Schams *et al.*, 1984; Bruckmaier *et al.*, 1994b).

In this study relatively large fractions of milk being left behind within the alveolar region when the animals were not adequately stimulated could have resulted in disturbed or inhibited milk ejection (paper II, III and IV).On account of the lesser drainage of alveolar milk into the cisterns between milkings in buffaloes, the proportion of residual milk could have a more potent effect through FIL than in dairy cattle, sheep and goats. It is known that FIL is most active when in close proximity with the apical membrane in the secretory epithelial cells. Similarly there exists an inverse relation between the rate of milk secretion per unit amount of mammary tissue and the fraction of residual milk (Peaker & Blatchford, 1988; Knight *et al.*, 1994). The contribution of insufficient milk ejection, small cistern, higher amount of residual milk, and effect of FIL could be reasons for a rapid drop in milk yield reported in buffaloes. This is know to happen due to repeated failure in milk ejection due to the death of the suckling calf or other reasons (for review see Pathak, 1992; Cockrill, 1974a).

We found that the cisternal area size and the cisternal milk fraction were higher in older animals than animals in early lactation stages (paper I). This has been reported in cattle where the intramammary pressure and udder fill in early stages of lactation while in older animals, the size and fraction are higher due to the tissue stretching effect (Bruckmaier & Blum, 1992; Bruckmaier & Hilger, 2001). The commencement of milk flow in response to induced milk ejection with physiological levels of oxytocin was also earlier in the early lactation stages.

Due to the small cisternal area and fraction, buffaloes could be good candidates for more frequent milkings, than milking two times a day. It is reported that cattle with small cisterns respond better to more frequent milkings (Dewhurst & Knight, 1994). Increasing the frequency of milking, from two to three times a day caused a 9–10% increase in milk yield in buffaloes (Dash, Basu & Sharma, 1976; Ludri, 1985). In cattle it has been reported that for the same amount of secretory tissue, cows with larger cisterns produce more milk and could endure longer intervals between milkings without a drop in milk yield than those with small cisterns (Knight & Dewhurst, 1994).

Availability of the cisternal fraction also has a direct influence on the milking strategy employed while machine milking. In buffaloes in the current study (paper II, III and IV), Mediterranean buffaloes of Egypt, and for those in Azerbaijan, commencement of milk flow while machine milking was not immediate as seen in cattle, sheep and goats (Marathe & Whittlestone, 1958; Aliev, 1969; Alim, 1982; Lind, Ranade & Thomas, 1997). The very small cisternal fraction was also the reason why no interrupted milk flow (bimodal) curves were observed even when buffaloes were milked without pre-stimulation (paper II). The close correlation between time until increase in oxytocin concentrations over a threshold level and commencement of milk flow also indicated that milk flow in buffaloes commenced only after oxytocin concentrations were elevated (paper III). Thus, in the light of what has been discussed earlier to prevent milking on empty teats, it is important to ensure that there is a good milk ejection prior to machine milking.

Heritability of the partitioning of milk in the udder has been established for decades and dairy cattle have been bred for higher cisternal fraction, smaller teat dimensions, and high milk flow rates (Johansson & Korkman, 1952). It has been postulated that there could be further selection on the udder storage capacities in dairy cattle. Animals will be selected for more frequent milking to suit the recent developments in automatic milking in some parts of the world like Europe. In other parts of the world like Australia and New-Zealand where cattle are on pasture they will be selected for larger udder storage capacities and longer milking intervals (Knight, 2001). Although buffaloes in India have been selected for milking characteristics, it possibly has not been done from a machine milking perspective. Apart from this, limited application of advanced breeding techniques like artificial insemination, has slowed down the process of breed improvement in this species (for review see Sastry, 1983).

Pre-milking stimulation, milking routines and milk removal

Intensive milk production and machine milking has put new demands on milk removal with requirements of shortest time for machine milking, suitable milk flow rate and maintenance of good udder health. In the current study when comparing different tactile stimulation it was observed that one minute manual pre-stimulation produced the best results in terms of milk ejection, average and peak milk flow, milking time and stripping yield (paper II). It was also seen that feeding along with manual pre-stimulation gave better results in these milking characteristics (paper III). However, while milking with only tactile stimulation of milking machine, milk ejection took longer, milk yield was significantly lower and stripping yield was significantly higher than during manual pre-stimulation (paper II, III). This indicates that in the absence of the cisternal fraction, milking on empty teats may causes discomfort to buffaloes and so manual pre-stimulation is important while milking buffaloes. In cows tactile stimulation of the machine is sufficient although it may result in lower increase in oxytocin, however, it had no influence on the milk yield (Mayer *et al.,* 1985; Bruckmaier & Blum, 1996). In this study the tactile stimulation of machine milking at lower vacuum levels during Duovac milking resulted in similar machine milk yield as the manual pre-stimulation. The advantage of tactile stimulation of machine milking at low vacuum was demonstrated in cows as well, where it resulted in higher machine yields and total milk yields (Grimm, 1992; Worstorff *et al.*, 1980). Apart from this during Duovac milking at low vacuum the "relief phase" is longer and this as discussed earlier is more efficient in removing the accumulation of tissue fluids at the teat tip under vacuum.

Induced milk ejection with oxytocin injection at physiological levels was not better than manual pre-stimulation's in terms of milking characteristics although it was significantly better than machine milking without manual pre-stimulation. It is well known although not documented that on large commercial farms oxytocin is widely used for inducing milk ejection in buffaloes. This was also reported in the survey among hand milked farms in India by Varma & Sastry, (1994) where it was seen that 13% of farmers used injectable oxytocin for inducing milk ejection. Persistent use of oxytocin could also result in addiction to this treatment as has been found in cows (Bruckmaier, 2003).

In this study it was observed that stripping yield as a fraction of the total milk yield and fat percent was significantly lower while milking without manual pre-stimulation or feeding during milking (paper III). Since the fat content in the milk is increasing during milking (Johansson, Korkman & Nelson, 1952), the effect of udder emptying can be seen in the fat content of the strip milk (Samuelsson *et al.,* 1993). It has also reported in cows that by excluding machine stripping from the milking routine increased milk yield loses to about 10% and also increased the incidence of udder infections (Ebendorff *et al.*, 1987).

Milking-related release of hormones

For a long time it has been acknowledged that milking is a complex process. It wasn't until the middle of the twentieth century with the use of bioassay and later with the help of radioimmunoassays that milk-ejection was understood as a separate physiological process. Ely & Petersen, (1941) were among the first to demonstrate milk ejection in dairy cows. The neuroendocrine pathway has come to be accepted as the physiological basis for milk ejection according to the model suggested by Tucker & Lansing (1978) and later on verified by

Crowley & Armstrong (1992). Sparse motor innervation of the mammary duct system has been reported and could be instrumental in a short reflex arc called also the "tap reflex" whereby the myoepithelial cells respond by contracting to a small tap to the udder skin (Mepham, 1987c; Lefcourt & Akers, 1984; Zaks, 1962).

In buffaloes, milk ejection follows the same neurohormonal mechanism as in cattle, sheep, and goats where adequate stimulation cause an increase in blood oxytocin levels in one minute (paper III). It had been proposed earlier by Aliev (1969) that commencement of milk flow in buffaloes is delayed by about 1–2 min on an average after start of pre-stimulation. In the current study, the combined effect of manual pre-stimulation and in-parlour feeding caused the release of oxytocin in about one minute and it was followed by a corresponding milk ejection in about one minute after attaching the milking unit. Thus the small delay in commencement of milk flow in buffaloes is due to the absence of the cisternal fraction of milk where it takes about two min for the intramammary pressure to build and for commencement of milk flow during machine milking.

Milk ejection occurred with a small increase in oxytocin over the basal levels and there was a positive correlation between the time oxytocin remained elevated over the basal levels and the total milking time (paper III). In cattle as well the oxytocin increase over a threshold level and a continuous release of oxytocin during the entire milking is essential for complete milk removal (Schams *et al.,* 1984; Bruckmaier, 1994b).

Neural receptors that are densely located at the teat are sensitive to touch, temperature and pressure provided through tactile stimulations (Findlay 1966). Tactile stimulations of suckling, pre-stimulation massage and washing of the teats or pulsating movement of the liner during machine milking are know casue milk ejection in cattle (Akers & Lefcourt, 1982; Bar-Peled *et al.,* 1995; Gorewit & Gassman, 1985, Lupoli *et al.,* 2001). However stimulation's other than tactile stimulation like feeding during milking and presence of calf independently or in conjunction with tactile stimulation enhance oxytocin release (Akers & Lefcourt, 1982; Svennersten *et al*., 1995; Svennersten, Nelson & Uvnäs-Moberg, 1990; Lupoli *et al.,* 2001)

In the current study the combined stimulation of feeding during milking and manual pre-stimulation produced the most pronounced release of oxytocin with a higher corresponding milk yield compared to only machine milking or manual pre-stimulation (paper III). Whereas with only machine milking the oxytocin levels did not increase over basal levels during milking, while there was a remarkable increase during hand stripping (paper III). In cattle it has been reported that hand milking produced a more prominent release of oxytocin than the tactile stimulation of machine milking (Gorewit *et al.,* 1992). Svennersten *et al.* (1995) and Johansson *et al.* (1999) demonstrated that feeding during milking also enhanced oxytocin release. In cattle partial milk ejection and incomplete milk removal due to the release of insufficient amounts of oxytocin has been reported (Bruckmaier & Blum, 1996; Bruckmaier *et al.,* 1996). As these animals were accustomed to the pre-milking routines of manual pre-stimulation and feeding during milking it is also possible that when they were deprived of both these, this could have caused discomfort and stress. As discussed it could also be due to milking on empty teats with a small cisternal fraction which could have led to inhibition of milk ejection.

The probable inhibition of milk ejection in buffaloes during milking in this study was not at the peripheral level which was evident from the immediate milk ejection which occurred with intravenous oxytocin at physiological levels (paper II). This was also evident from the non-availability of the alveolar fraction of milk during oxytocin receptor blockade where only a supra physiological dose of oxytocin could extract it (paper I). Thus the failure of milk ejection in buffaloes is caused by an inhibition of oxytocin release at the central level. In cattle it has been clearly demonstrated that the main cause of lack of milk ejection was because oxytocin was not released in response to pre-stimulation and milking (Bruckmaier *et al.,* 1993; Bruckmaier, Schams, & Blum, 1994b; Bruckmaier *et al.,* 1996; for review see Bruckmaier & Blum, 1998).

In the study it was demonstrated that even small changes in the milking routines influences milk ejection and milk removal. Reduced in parlour feeding of concentrates also probably caused an increase in oral behaviour as the animals searched for food which could have led to stress (paper IV). Thus frequent change in milking routine should be avoided in buffaloes as this could lead to a drop in milk yield as discussed earlier. Even in cattle changes in milking routines like milking in unfamiliar condition is known to influence milk ejection (Bruckmaier *et al.,* 1996; Bruckmaier *et al.,* 1993). However, due to the small cisternal drainage of milk the magnitude of autocrine inhibition of milk secretion could be more severe in buffaloes.

The simultaneous milking related release of prolactin and cortisol preceded by oxytocin was observed only in the combined stimulations of feeding during milking and manual pre-stimulation. That these hormones are released during simultaneous feeding and milking has also been reported in cattle (Johansson *et al.,* 2000). The mechanism of how oxytocin influenced the release of prolactin and cortisol is well documented and it could be the same in the case of buffaloes as well (Legros, Chiodera & Greenen, 1988; Mori *et al.,* 1990; Cook, 1997; Petersson, Hulting & Uvnäs-Moberg, 1999). However, it is not clear how prolactin concentration remained higher during probable incomplete milk ejection and inhibition of oxytocin release while cortisol levels remained at the basal levels (paper III). It has been known that increase in prolactin concentrations can be stress related in rats as well as in cattle and sheep (Ronchi *et al.,* 2001; Matthews and Parrott, 1992; Troner *et al.,* 2002; Johnson & Vanjonack, 1975). Stress of parturation is reported to increase prolactin concentrations in buffaloes (Batra, Phawa & Pandey, 1982; Arya and Madan, 2001). In this study while milking without feeding or pre-stimulation the cortisol concentrations remained constant simultaneously with low levels of oxytocin, indicating that the buffaloes might have been exposed to some kind of stress. It has been observed in sheep that following acute stress prolactin might depress cortisol levels (Cook, 1997). However, it is necessary to study the inhibition of milk ejection in buffaloes to get a clearer understanding of the physiological affects it has on milk synthesis and persistency of lactation in buffaloes.

Maintenance behaviour in a mechanised system

Buffaloes adapt very well to a management system with mechanisation of the activities like concentrate feeding, feeding of water, manure handling and milking (paper IV). Time budgets and diurnal variations of the main behavioural activities like eating, rumination, resting and standing were comparable to cattle under similar conditions and was similar to reports regarding buffaloes in non mechanised conditions (Grant & Alright, 1995; Hafez, 1975; Odyuo, *et al.*, 1994).

Buffaloes are prone to thermal stress owing to their dark skin and fewer sweat glands per unit skin area and it has been reported that thermal stress directly effect feed intake (Sastry & Tripathi, 1998; Thind & Gill, 1986). In earlier studies it was reported that buffaloes ate more intensively in the morning and the cooler parts of the day. However, in this study the buffaloes ate most intensively in the hottest parts, which could partly be due to the cooling effect of the water sprinkler and the ceiling fans kept on in between 12.00 to 14.00 h.

Increased access to roughage altered the diurnal variations of some behaviour seen under conditions of lesser access to roughage. Eating behaviours showed no significant diurnal variations while time spent standing idle and standing and ruminating was shorter and time spent sleeping was longer with conditions of increased access to roughages. This is similar to what is reported in cattle where feeding has priority over rumination and under restricted feeding condition animals cattle more standing in anticipation of being fed (Albright & Arave, 1997). Thus, animals should have access to roughage through out the 24-h period as this will improve the resting behaviour and under confined conditions feed accessibility is know to reduce competition and improve feed intake (Albright & Arave, 1997).

Influence of milking management and herd size on udder health

In this study (paper V) 97.5 % of the quarters did not show any clinical signs of mastitis and hence the prevalence referred to in this study is predominantly the subclinical form. Prevalence based on SCC was comparable to prevalence based on bacteriological results, which indicates that SCC well reflects infectious mastitis. Prevalence based on bacteriological positive results was similar to that reported by Chander & Baxi (1975) and Bansal *et al.*, (1995).

Milking management systems had the greatest influence on udder health followed by the herd size and the interaction of management and herd size. Hence, udder health and occurrence of mastitis in buffaloes is highly related to management and to characteristics related to the size of the herd, as is the case for dairy cattle (Carroll, 1977; Ekman 1998). CH herds had the lowest prevalence of mastitis as measured by each inflammatory indicator and in small herds also by infection. In the MH herds, the prevalence was higher which indicates that calf suckling and not hand milking is the major contributor to good udder health in the CH herds. As discussed earlier, calf suckling is known to lead to a more intense oxytocin release and milk ejection (Akers & Lefcourt, 1982; Bar-Peled *et al.*, 1995; Lupoli, 2001). Calf suckling is also known to lower the number of bacteria on the teat skin (Rasmussen & Larsen, 1998). A well-emptied udder is less prone to infection.

Machine-milked large herds had the highest prevalence of mastitis. However, in the small herds with MM, the prevalence was markedly lower, and based on SCC and proportion of neutrophils even lower than the prevalence in the small herds with MH. Hence, it appears that it is not the machine milking as such that is negatively influencing udder health. The reason for impaired udder health in the large machine-milked herds may be found in an inappropriate condition or use of the milking machine. This is known to impair the udder health in cows (Carroll, 1977; Ekman 1998). In this study, most small herds with milking machines are family farms managed by the owners themselves without hired labour. It seems likely that the owner is anxious to get the most out of the investment in the milking machine with careful handling and use. The reasons behind the generally weaker udder health found in the large herds are probably the same as for cattle (Carroll 1977; Ekman 1998). These could include a diminished consistency in management routines because of more people being involved and less consideration being given to the individual animal. There is no obvious explanation as to why the small MH herds seem to have more problems with infections than the large farms in the same category. The generally higher frequency of infections with environmental bacteria in large herds agrees with our general impression that in the large herds, environmental and management hygiene was often neglected and not as good as in the small herds.

There is a general lack of important knowledge about proper milking routines, hygiene, and mastitis and about how to use milking machines correctly and hygienically among farmers and especially among milking personnel. This is most probably the main reason behind the occurrence of mastitis in the present study, along with difficulties with housing practices that keep teats clean and dry. To improve the situation, efforts should be put into the advisory service and education of farmers and milkers.

The cell types found in this study in buffalo milk are similar to those reported in cows and generally have the same proportion in samples with low and high SCC as reported in cows (Hageltorn & Saad, 1986; Östensson, 1993a, 1993b; Pillai *et al.*, 2001). This also agrees with what has been reported earlier in buffaloes by Dhakal, (1992). However, an interesting observation is that some milk samples in the present study had a high content of cell fragments which have also been reported in goat and camel milk (Wooding, Peaker & Linzell, 1970; Abdurahman *et al.*, 1992). In these species it was considered to be due to the apocrine milk secretory process. The background to the presence of the fragments found in buffalo milk can not yet be explained but requires further studies. This will help in understanding the mechanism of milk secretion in buffaloes.

Although there was a good agreement between the mean SCC for each CMT score in buffalo milk in this study and the values given in bovine milk (Schalm, *et al.*, 1971) the correlation between SCC and CMT was low. This implies that CMT should be used with some caution in estimating the somatic cell content in buffalo milk. In general, the average proportion of neutrophils was increasing with increasing SCC but the correlation between these parameters appeared to be weaker than for bovine milk (Kelly *et al.*, 2000; Pillai *et al.*, 2001), even if a low correlation also for bovine milk with low cell counts has been reported (Östensson, 1993a). The results might indicate that the SCC in buffalo milk may be more influenced by reactions, in which other kinds of leukocytes than neutrophils play the major role. To clarify this requires further studies.

Conclusions

- In buffaloes, teats are longer, thicker, and have longer teat canals than reported in dairy cows. Teats are collapsed and limp and relatively empty prior to milk ejection as they contain only a small fraction of milk. When milk ejection occurs there is a distinct increase in teat length and thickness and the cisternal area.
- Buffaloes have relatively smaller cisterns compared to dairy cattle, sheep, and goats. The cisternal fraction is only 5% of the total milk yield. Thus, during machine milking, the milk flow does not commence unless the alveolar fraction of milk is available.
- Among the different types of tactile stimulation techniques used, manual pre-stimulation induces faster milk ejection and better emptying of the udder. However, a tactile stimulation by the milking machine at lower vacuum levels and with a longer relief phase of pulsation also led to a comparable emptying of the udder.
- Milking-related release of oxytocin, prolactin, and cortisol occurs spontaneously in response to adequate pre-stimulation and this neurohormonal reflex in buffaloes is similar to that observed in other species.
- The combined stimulation of feeding during milking and manual pre-stimulation causes a more intense oxytocin release, faster milking, and better emptying of the udder with a lower strip yield compared to only manual pre-stimulation or only machine milking.
- Small changes in milking routines are sufficient to cause disturbances in milk ejection in buffaloes. This is reflected in the behaviour during milking and the milk yield.
- Buffaloes adapt very well to an environment with a relatively higher level of mechanisation without disturbing their natural rhythm and behaviour. However increased access to roughage could have a positive influence on the maintenance behaviour of buffaloes that results in increased resting and sleeping behaviour.
- Udder health and occurrence of mastitis in buffaloes was strongly influenced by the milking management system and to some extent also by the size of the herd.
- Small herds with a combination of calf suckling pre-stimulation and subsequent hand milking had the lowest prevalence of mastitis, that may be a result of better emptying of the udder in the animals after calf stimulation.
- Large machine-milked herds had the highest prevalence of mastitis while the prevalence in the small machine-milked herds was markedly lower. Hence, machine milking, *per se*, appears not to be the reason for impaired udder health but rather inappropriate condition, maintenance and use of the machine.
- The cell types found in buffalo milk are the same as have been reported in cows and are present in approximately the same proportions as in cow milk in samples with low and high SCC, respectively. However, the correlation between CMT, SCC and proportion of neutrophils in buffalo milk appears to be weaker than in bovine milk. This, and to identify the origin of the cell fragments found in buffalo milk, requires further studies.

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