Keeping Goats and Kids Together

Focus on Milk Ejection, Milk Composition, Curd Yield and Animal Welfare

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Abstract

Milk with high concentrations of fat, total protein and casein is required for cheese production. The Swedish landrace goat produces milk with relatively low dry matter content. The explanation for this may be genetic factors in combination with unfavourable management and milking routines. Goats have a large udder cistern and can therefore be milked without oxytocin (OT) stimulated milk ejection that enables fat rich alveolar milk to be available for milking. Earlier studies have shown that vasopressin (AVP) also can increase fat content in goat milk. The overall aim in this thesis was to investigate if cheese processing properties of Swedish goat milk could be optimized by suckling. Analyses of milk from 28 Swedish goat herds showed that 65% had low concentrations of α_{S1}-casein and only 12 % had high expression (Paper I). In Paper II it was demonstrated that AVP and OT increase simultaneously during suckling but not during hand milking. The importance of AVP was followed up in paper III, and it was demonstrated that AVP administration can cause milk ejection similarly to OT. In paper IV it was found that milking management systems, such as suckling before milking, increased milk fat concentration compared to milking before suckling. When dams and kids were together for a longer time (16h) milk fat, casein concentration and individual curd yield (%) were higher compared to when they were together for shorter time (8h). Thus overall, keeping goats and kids together is beneficial for animal welfare, increases milk fat concentration and curd yield, and can thereby improve cheese production.

Keywords: casein, fat, goats, milk ejection, oxytocin, suckling, vasopressin

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Dedication

To the Släsby goats: Nitti, Zaxalax, Inga, Gerda, Krusa, Lallanko & Flåset

And to the SLU goats: KÄCK, Pim Pim, Zoo, Polly, Fruxo, Keso, Nogger, Biskvi, Persika, Singoalla, Passion, Plommon, Sharon, Kiwi, Topas, Turmalin, Kefir, Latte, Loranga, Oboy, Jet, Puma, Split, Svea, Mozzarella, Semla, Struva Korint, Maräng, RedBull, Tapiren, Champange, Soda, Stout, Sprite, Kahlua, Citrin , Safir, Jaspis, Storstrut, Solero, Åska, Storm, Odon, Glada, Duva, Solbritt, Hjortron, Baileys, Calippo, Korint, Caprin, Mango, Litchi, Guava, Lapsang, Lärka, Trana, Knäck, Top Hat, Sitting Bull, Magnum, 88:an, Puck, Frix, Spirello, Tip Top, Trollis, Solero, Igloo, Israket, Kesella, Grädde, Kelda, Bregott, Bärry, Becel, Verum, Valio, Lätt, Lagom, Halloumi, Joggi, Milko, Arla, Pepino, Druva, Lime, Aprikos, Melon, Kokos, Tamarillo, Mandarin, Apelsin, Citron, Ananas, Brago, Kubb, Cockie, Rån, Kex, Mazarin, Muffins, Napoleon, Princess, Ballerune, Cornetto, Twister, Piggelin, Calippo, Hula Hula, Solsting, , Kristall, Bandit, Opal, Bov, Svarte Petter, Pirat, Tjuv, Snut, Ametist, Skurk, Rövare, Fjällräven, Levis, Tiger, Diesel, Prada, Armani, Dior, Lacoste, Only, Peak, Brooks, Mexx, Chevre, Brie, Gaja, Balder, Zeus, Eros, Aurora, Athena, Luna, Fortum, Tor, Pan, Hades, Nike, Nyx, Birk, King, Klang, Katla, Skorpan, Skrållan, Melker, Madicken, JumJum, Ronja, Mattis, Lovis, Myran, Loppan, Hermelin, Chinchilla, Sparven, Lo, Lemur, Björn, Humla, Tiger, Kobra, Krypet, Panda, Okapi, Foliora, Dhol, Grävling, Dingo, Vessla, Kanin, Viktor, Bockilocki, Kruuse, Gösta, Silver, Geting

And to all goats on the Swedish farms

De riktigt stora människorna är de som får andra att känna sig stora….!!
Gethallen

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Johansson, M., Högberg, M. & Andrén, A. (2014). High frequencies of the α_{S1}-casein zero variant in milk from Swedish dairy goats. *Sheep and Goats Research Journal* 29, 1-4.

II Olsson, K. & Högberg, M. (2009). Plasma vasopressin and oxytocin concentrations increase simultaneously during suckling in goats. *Journal of Dairy Research* 76, 15-19.

III Högberg, M., Olsson, K. & Dahlborn, K. (2014). Can vasopressin induce milk ejection in the lactating goat? *Small Ruminant Research* 121, 111-115.

IV Högberg, M., Dahlborn, K., Hydbring-Sandberg, E., Hartmann, E. & Andrén, A. (2016). Milk processing quality of suckled/milked goats. Effects of milk accumulation interval and milking regime. *Journal of Dairy Research* 1-7.

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1 Introduction

The domestic goat (*Capra hircus*) is a sociable, inquisitive and intelligent species that was first domesticated ca. 10 000 years ago (Miranda de la Lama & Mattiello, 2010). Today, the goat population in the world is approximately 860 million animals. The production and consumption of goat's cheese has increased significantly in recent decades (Silanikove *et al*., 2010) and the world production of goat milk is more than 15 million metric tonnes (FAO, 2008). In Sweden, the number of dairy goats has more than doubled in recent years and locally produced goat's cheese made in on-farm dairies has become very popular. When producing cheese, milk with a high concentration of fat, total protein and casein is required, since these components have a major impact on both the quality and yield of the curd (Clark & Sherbon, 2000; Soryal *et al*., 2005; Damian *et al*., 2008).

Swedish dairy goats are among the highest yielding goat breeds in the world and with good care and feeding, they can produce 1000-2000 litres of milk per year (SJV, 2015a). However, compared with some other breeds, the milk has a relatively low concentration of fat, total protein and casein, which can affect the cheese making properties negatively. One of the reasons for this difference might be the genetic protein profile. The Swedish goat is closely related to the Norwegian breed, which recently was shown to have a high number (70%) of individuals with a less successful genotype for cheese making (Devold *et al*., 2010). Therefore, the first aim in this thesis was to to provide a survey of the Swedish goat population with the aim to estimate the frequency of Swedish goats producing low levels of α_{S1}-casein (CN).

Another way of improving curd yield is to increase the fat concentration in the milk. The udder is divided in two parts, the alveolar and the cisternal compartment. The cisternal milk can be easily obtained without oxytocin induced milk ejections. Each time an offspring suckle a neuro endocrine reflex occurs that transport the fat rich milk from the alveolar part to the cisternal part. This is called milk ejection and the hormone involved is oxytocin. The relative size between the alveolar and cisternal compartment varies between species and breeds. In species with a small cisternal part (e.g. horse, camels, buffaloes) it is most common to let the calf/foal stimulate the udder to receive milk ejection. In dairy cattle, oxytocin can be released by stimulating the udder without attending the calf. The goat has a large gland cistern (40-80%, Marnet & McKusick, 2001) and this milk is available for milking without the milk ejection reflex. The cisternal milk has a lower fat concentration than the alveolar milk and thus, insufficient milk ejection can be one explanation why Swedish dairy goats often produce milk with low dry matter content. Suckling might be a more potent stimulus to milk ejection than milking (Delgado-Pertinez *et al*., 2009a), it was therefore of interest to investigate whether the milk fat concentration could be increased by suckling.

Dairy goats can be kept in intensive, semi-intensive or extensive management systems (FAO, 2014). In intensive systems, which are most common in industrial countries, early weaning (within a few days) of the offspring is customary (Miranda de la Lama & Mattiello, 2010). Along with the increased intensification, demand for food produced with respect of animal welfare has become one of the most debated issues in the field of animal husbandry (Battini *et al*., 2014).One debated issue is for example the early separation between mother and offspring, which is believed to be the best way of obtaining the largest amount of milk for processing and consumption. Early separation between mother and offspring may compromise welfare, and can be stressful for both goats and kids (Boivin & Braadstad, 1996; Bergamasco *et al*., 2005; Newberry & Swanson, 2008; Miranda de la Lama & Mattiello, 2010; Winblad von Walter *et al*., 2010).

However, in semi-intensive or extensive management systems, it is more common that dam and offspring are kept together for longer periods (Marnet & McKusick, 2001). In Sweden, where both intensive and semi-intensive systems exist, the separation between dam and offspring can occur from a few days to several months (Brandt, 2009). It is of great importance to investigate how alternative management systems based on both milking and suckling (mixed management) affect milk production in dairy goats. It has for example been shown that partial suckling regimes can increase the commercial milk yield in dairy ewes (Knight *et al*., 1993; McKusick *et al.,* 20001) and in dairy goats (Delgado-Pertínez *et al*., 2009b).

On the other hand, it has been reported that ewes kept in mixed management systems produce milk with a poor fat content, which is explained by impaired milk ejection during milking (McKusick *et al*., 2002a; Jaeggi *et al*., 2008). The overall aim in this thesis was to investigate if cheese processing properties of Swedish goat milk could be optimized by suckling.

2 Background

2.1 The Swedish Landrace Goat

The dominant goat breed in Sweden is the Swedish Landrace. This breed is believed to originate from three different native ‘sub-breeds’, the Jämt goat, the Lapp goat and the Göinge goat, of which only small numbers exist today. However, maintenance plans are in place for preservation of all these endangered breeds. The Swedish Landrace goat is closely related to the Norwegian Landrace goat, since breeding animals have been continually moved across the border over the years (SJV, 2015a). The milk quality characteristics are therefore similar in the two breeds. The milk from Swedish Landrace goats is mainly used for cheese processing. In Sweden, there are no dairy associations receiving goat’s milk and therefore the cheese is produced on-farm in private dairies. In these types of businesses the workload can often be very high, as it involves *e.g*. animal care and milking management in combination with cheese processing, marketing and selling the produce. Demand and consumption of goat’s cheese have both increased significantly in recent decades. The number of dairy goats in Sweden has more than doubled in the past few years (to about 14 000 goats, SJV, 2015b) and locally produced goat’s cheese has become very popular. There were 96 dairy goat farms registered in 2014 (SJV, 2014). The Swedish Landrace is one of the highest-yielding goat breeds in the world. With good care and feeding, the Swedish Landrace can produce between 1000 and 2000 litres of milk per year (SJV, 2015a). However, the milk has relatively low concentrations of fat, protein and casein (Sjödin, 1979; Högberg, 2011b), which influences the cheese-making properties negatively, since high concentrations of these components are required for both quality and yield of curd (Pirisi *et al*., 1994; Clark & Sherbon, 2000).

Along with the rising demand for locally produced foods, concerns about ethical issues regarding how food is produced with respect to animal welfare have increased. One debated ethical issue in Sweden is the early separation of mother and offspring and the killing of male kids early in life (Sveriges Radio, 2015).

2.2 Management Systems for Dairy Goats

Dairy goats can be kept in intensive, semi-intensive or extensive management systems (FAO, 2014). In intensive systems, early weaning of kids is customary and is believed to be the most profitable way of increasing milk supply for dairy products (Sevi *et al*., 2009 Miranda de la Lama & Mattiello, 2010). In semi-intensive or extensive management systems, it is more common for dairy goats and sheep to be kept together with their offspring for longer periods. During the suckling period (usually 30-60 days in dairy ewes), dams are often suckled by their offspring exclusively or partially (Marnet & McKusick, 2001).

One way to save more milk for the dairy if mother and offspring are kept together is to practise partial suckling. It has been shown that this type of regime can influence milk production positively in dairy sheep (Knight *et al*., 1993; McKusick *et al*., 2001; McKusick *et al*., 2002a; Rassu *et al*., 2015; Tzamaolukas *et al*., 2015) and in goats (Peris *et al*., 1997; Hernandez *et al*., 2007; Delgado-Pertínez *et al*., 2009a, 2009b). Delgado-Pertínez *et al*. (2009b) demonstrated that dams kept with their kids for 18-20 h daily had higher milk production during the entire lactation (210 days) than dams that were only milked and with artificially reared kids. However, Peris *et al*. (1997) found no difference in milk production between naturally suckled dams (separated from their kids for 6 h in daytime) and dams milked only. In a similar study on sheep, there was no difference in milk production between ewes kept without lambs and ewes kept in a mixed system with daily separation from their lambs for 15 hours (McKusick *et al*., 2001). In mixed regimes, dams and offspring can be kept together either during daytime or at night, since there is reportedly no diurnal difference in milk production between these two systems (Knight *et al*., 1993). Furthermore, management systems involving both milking and suckling can be advantageous for on-farm cheese makers, since according to McKusick *et al*. (2002a) the workload can be reduced by 27% if bottle feeding with colostrum and milk replacer is exchanged for natural suckling. In Sweden, it is not always customary to separate dams from kids early in life. Goats are kept in both intensive and semi-intensive systems and the separation between dams and kids occurs from a few days to several months (Brandt, 2009).

2.3 Milk synthesis and milking frequency

The mammary gland grows during pregnancy (mammogenesis). Before parturition, the mammary cells differentiate to start milk secretion (lactogenesis). Complete removal of the alveolar milk by either milking or suckling maintains milk synthesis and ongoing lactation (galactopoiesis). Finally, the mammary gland regresses after drying off (involution) (Knight & Wilde, 1993).

Milk yield and the shape of the lactation curve are determined by the number of secretory cells. Milk synthesis is dependent on endocrine factors that affect the entire udder and on local factors that affect a particular udder half. Maintained milk synthesis depends on frequent milk removal, and milk production eventually stops if milk removal ceases. This effect has been attributed to a combination of inhibitory effects from the presence of milk in the secretory tissue and high intra-mammary pressure. The effects of the pressure that milk fill exerts on secretory cells and blood perfusion in the capillary net has been discussed since Fleet and Peaker (1978) first found that the rate of milk secretion declines when the calculated pressure within the alveoli increases. Several studies have shown that more frequent milking increases milk yield in goats (Henderson *et al*., 1985; Wilde *et al*., 1987; Koyuncu & Pala, 2008), which leads to mammary growth and increased activity per cell. In contrast, less frequent removal of milk inhibits further milk synthesis (Wilde *et al*., 1987; Li *et al*., 1999; Fitzgerald *et al*., 2007). Moreover, when milk is present in the secretory tissue the number of cells in the alveoli decreases, which is an effect of apoptosis (Li *et al*., 1999; Knight, 2001). Less removal of milk results in reduced blood flow to the udder, which can initiate apoptosis (Capuco *et al*., 2003; Koyuncu & Pala, 2008). Local mammary factors regulate milk synthesis in each gland (left or right in goats) and they are independent of each other. If milking frequency increases in one gland only, milk yield will increase in that gland, but not in the other (Stelwagen, 2001).

Wilde *et al*. (1996) suggest that the autocrine peptide feed-back inhibitor of lactation (FIL) is another factor responsible for the inhibitory effect during incomplete milking. FIL is only present in the alveolar compartment and is proposed to inhibit milk secretion without influencing gross milk composition (Wilde *et al*., 1996). When milk is accumulated in the alveolar fraction, inhibition of further milk synthesis arises (Marnet & Komara, 2008). Frequent milking in goats has been shown to reduce the effect of FIL (Peaker & Wilde, 1996) and milk must be removed from the alveoli to prevent inhibition of milk synthesis (Henderson & Peaker, 1987). Cessation of milk removal leads to rapid changes in mammary secretion and initiation of the process of active mammary involution. From another point of view, Silanikove *et al*. (2000) proposed that a local mechanism which connects the plasmin-plasminogen system is responsible for the autocrine inhibition of lactation. This proteose-peptone channel blocking (PPCB) inhibits the ion channels in mammary epithelia apical membranes and inhibits lactose and monovalent ion secretion (Na^+, K^+ and C^-), which results in decreased milk volume.

2.3.1 Milking interval

Many studies have investigated how different milking intervals affect milk yield in goats. As noted above, increased milking frequency enhances milk yield and stimulates mammary cell activity and secretory cell numbers (Knight & Wilde, 1993). It has also been found that three daily milkings in one gland increased the weight of that gland compared with the other gland, which was milked twice daily, probably due to a reduced rate of regression of secretory cells in the alveoli (Henderson *et al*., 1985). A study by Salama *et al.* (2004) demonstrated that cisternal cavity area did not differ significantly between goats milked once or twice daily. It has also been shown that within the same udder, mammary enzyme activity (acetyl-CoA carboxylase, fatty acid synthetase and galactosyltransferase) is higher in glands milked three times daily than in glands milked twice daily (Wilde *et al*., 1987).

On comparing once or twice daily milking, conflicting results have been found and the effects of extended milking intervals have been shown to vary depending on species, breed and genetic profile of animals (Marnet & Komara, 2008). For example, the decrease in milk yield with once daily milking has been shown to vary between 6% in the Tinerfena breed and 40% in the French Alpine breed (Table 1). When switching from twice to once daily milking managements, the cisternal size seems to have major effects upon milk losses in once daily milking management. For example, Saanen dairy goats, which have a small cistern, have been shown to display milk losses of 26% when milked once daily (Marnet & Komara, 2008). Muriciano-Granadina dairy goats, categorised as having medium cistern size, had milk losses of 18% in that study, whereas the large cistern Canadian dairy goats only had a milk losses of 6% with a change from twice to once daily milking (Marnet & Komara, 2008).

The once daily milking system could be a beneficial option for dairy goat farmers producing cheeses in on-farm dairies by lowering the otherwise heavy workload (animal care, milking, cheese processing, storage of cheese, marketing and selling).

Table 1. *Decrease in milk yield during once- daily milking compared to twice daily milking in different goat breeds.*

Decrease in Milk yield (%)	Breed	Reference
6%	Tinerfena*	Capote *et al.*, 2006*
6-7 %	Damascus	Papachistoforou *et al.*, 1982
6-8 %	Canarian	Capote *et al.*, 1999
15%	Alpine	Marnet *et al.*, 2005
18%	Muricano Granadina	Salama *et al.*, 2003
21%	Muricano Granadina**	Salama *et al.*, 2004*
26%	British Saanen	Wilde and Knight, 1990
26%	Saanen	Boutinaud *et al.*, 2003
21-40 %	Alpine	Mocquot, 1980
36%	Alpine	Mocquot *et al.*, 1978

*Source: Modified version of a table in Marnet & Komara (2008). *from Capote* et al. *(2006) **from Salama et al. (2004).*

2.4 The neuroendocrine milk ejection reflex

The udder is innervated by two types of nerves, sensory fibres (afferent) in teats and skin and sympathetic fibres (afferent). The milk ejection reflex during suckling and milking is a neuroendocrine reflex that has both an afferent pathway (conducted from teats to the brain via neurons) and an efferent pathway (conducted from pituitary to the mammary gland via blood-borne hormones). Pressure from suckling or milking activates sensitive teat receptors, which create nerve impulses that reach the hypothalamus and release oxytocin (OT) from the pituitary to the blood (Bruckmaier & Blum, 1996). Oxytocin is then transported to the mammary gland and binds to OT receptors on the myoepithelial cells that surround the alveoli like a basket. The myoepithelial cells contract and the alveolar milk is released into the cisternal area by active expulsion, by a process called the milk ejection reflex (Bruckmaier, 2005). For complete milk removal, milk ejection is necessary throughout the entire milking process (Bruckmaier *et al*., 1994a). As early as 1910, Ott and Scott reported that extract from the posterior pituitary gland induced milk ejection in the goat, while a later study showed that it was OT that controls milk let-down

(Ely & Petersson, 1941). In addition, different kinds of tactile stimulation of the mammary gland cause different OT responses. For example, suckling has been shown to be a more potent stimulus than milking (Marnet & Negrao, 2000; Delgado-Pertinez *et al*., 2009b).

2.5 Milk Storage in the mammary gland

The goat udder is divided into two separate and independent glands, each with its own secretory tissue and cisternal cavity and each drained by a separate teat (Bruckmaier & Blum, 1996). Milk is produced in the secretory tissue in the alveoli and in ruminants this milk is stored in two udder fractions, as cisternal and alveolar milk. The cisternal milk is located in teats, gland cistern and large milk ducts. The alveolar milk is found in small ducts and alveoli, which are surrounded by myoepithelial cells with OT receptors (Cross, 1977). The proportion of milk stored in the cisternal and alveolar fractions varies according to species, breed, age, stage of lactation and milking interval (Marnet & Komara, 2008). In contrast to cows, goats have large gland cisterns, where between 40-80% of the milk can be stored (Marnet & McKusick, 2001). In the dairy cow, most of the milk (80-100%) is stored in the alveolar fraction (Bruckmaier, 2005). The cisternal milk can be easily obtained without a neuroendocrine milk ejection reflex, while milk stored in the alveolar fraction can only be expressed into the cistern after contraction of the myoepithelial cells (Andersson, 1951; Soloff *et al*., 1980; Bruckmaier and Blum, 1994; Marnet & McKusick, 2001).

Moreover, a larger cistern plays an important role in storage of milk between milkings, which further affects the removal of milk during milking (Marnet & McKusick, 2001). In goats, large volumes of milk can be obtained without milk ejection (Marnet & McKusick, 2001) and caprines are thus less dependent on milk ejection (Peris *et al*., 1997). Therefore, pre-stimulation for longer than 30 s is not necessary during machine milking of goats (Basic *et al*., 2009), but the pre-stimulation time required varies between breeds depending on differences in cistern size. In addition, animals with large cisterns are able to store milk more effectively between milkings (Marnet & McKusick, 2001). Greater storage capacity also leads to higher milk yield in goats and minimises the milk losses in once daily milking (Marnet & Komara, 2008).

While goats are less dependent on milk ejection, the action of OT is still of great importance for obtaining higher milk yield and higher fat content. Milk from the gland cistern has unfortunately a low fat concentration, since fat globules (especially large globules) are stored in the alveolar fraction and do not pass freely from the alveoli to the gland cistern (McKusick *et al*., 2002b). The fat-rich alveolar milk can be transported to the cisternal area only due to the action of OT (Bruckmaier & Blum, 1994). This explains why milk stored in the cistern has a low fat concentration and why milk from the alveoli is rich in fat (Linzell & Peaker, 1971). It also explains why the fat concentration is low in the beginning of milking and increases during the milking procedure. For example in cows, the first cisternal milk fraction can contain 2.6% fat, while the last fraction of residual milk can have a fat concentration of 12.8% (Sarikaya *et al*., 2005). Other milk components, like milk protein, lactose and casein, are small molecules and can pass freely between the alveolar and cisternal compartments. The concentrations of these components therefore remain quite constant during milking (McKusick *et al*., 2002b; Salama *et al*., 2005; Castilllo *et al*., 2008).

2.6 Plasma oxytocin and vasopressin during suckling and milking

Oxytocin (OT) and arginine-vasopressin (AVP) are two closely related neuropeptide hormones synthesised in the hypothalamus and stored in the posterior lobe of the pituitary gland. These two peptides can act as neurotransmitters in the brain and as hormones in the peripheral tissues, being able to bind to each other's receptors (Zingg, 1996), although with less affinity (Åkerlund et al., 1999). At low plasma levels, AVP acts as a water-saving hormone in the kidneys, while at high levels it is a vasoconstrictor increasing blood pressure. The main functions of OT are constriction of the smooth muscles in the uterus during parturition and stimulation of milk let-down. Moreover, OT plays an essential role in establishing the mother-offspring bond (Poindron *et al*., 2007).

In the mammary gland, both OT and AVP V1 receptor are found on the myoepithelial cells (Soloff *et al*., 1989). AVP has been shown to induce contraction of myoepithelial cells isolated from rabbit mammary glands (Lollivier *et al*., 2006) and intravenous infusions of AVP have been shown to increase milk flow and milk fat concentration in goats (Olsson *et al*., 2003). These results show that AVP can affect milk excretion, but the physiological role of AVP is still unclear. Reported changes in plasma AVP concentrations during milking are contradictory. During hand milking of goats, Seckl & Lightman (1988) found increased plasma OT concentration, but no change in AVP concentration. In another study, only plasma OT concentration was elevated during machine milking in cows and there was no change in the AVP concentration (Bruckmaier *et al*., 1992b). In rats, Suzuki *et al.* (2000) found that plasma OT and AVP concentrations both increased after suckling, and suggested that AVP was released to stimulate water reabsorption in the kidneys, saving water for the milk.

2.7 The protein profile in milk

The protein in milk from Swedish Landrace goats consists of 72 ± 5 % caseins and 28 ± 5 % whey protein (Högberg, 2011a, 2011b). The casein concentration is in general lower in caprine milk than in bovine and ovine milk (Bramanti *et al*., 2003; Park *et al*., 2007). Caseins are found as micelles containing colloidal calcium nanoclusters. The main biological function of the casein micelle is to deliver calcium and amino acids from mother to offspring (Sjaastad *et al*., 2003). When the proteins come into contact with gastric enzymes (chymosin), the caseins coagulate and the caseins and whey protein separate. The caseins are in a solid phase (curd), while the whey proteins are in a soluble phase (Walstra *et al*., 1999). The ability of casein to coagulate is therefore used in traditional cheese making, but can also be used to estimate the casein concentration in milk.

The casein fraction of goat's milk, like that of cow's and sheep's milk, consists of four components: α_{s1}-, α_{s2}-, ß- and ĸ-casein (CN). The whey protein consists of β-lactoglobulin, α-lactalbumin, immunoglobulin and serum-albumin (Walstra, 1999). The amount and proportion of milk casein vary between species (Thomann *et al*., 2008), breeds (Clark & Sherbon, 2000; Raynal-Ljutovac *et al*., 2008) and individuals. It is well known that goat's milk contains lower levels of α_{s1}-CN and higher levels of ß-CN than bovine and ovine milk (Walstra *et al*., 1999; Bramanti *et al*., 2003; Park *et al*., 2007), but the proportion varies between breeds and individuals (Clark & Sherbon, 2000). For example, milk from Nubian goats contains higher amounts of α_{s1}-CN and lower amounts of ß-CN than milk from Saanen goats (Moatsou *et al*., 2004). The variation in casein composition is mainly influenced by genetic polymorphisms on the α_{s1}-, α_{s2}-, *ß*- and ĸ-*CN* loci. The polymorphism at the caprine α_{s1}-*CN* locus has received considerable interest over the past few years, due to its impact on quality and important technological properties of the milk (Park *et al*., 2007). Today, as many as 18 different variants of caprine α_{s1}-CN have been identified (Caroli *et al*., 2007). Depending on their ability to produce α_{s1}-CN, goats can be classified into four classes; strong, medium, weak or zero (Park *et al*., 2007). Strong variants (A, B1, B2, B3, B4, Bk, C, H, L and M) synthesise α_{s1}-CN at a high level (3.5 g/L milk), medium variants (E and I) at a medium level (1.1-1.7 g/L), weak variants (F and G) at a low level (0.45 g/L) and zero variants do not synthesise α_{S1}-CN at all (Park *et al*., 2007). The strong goats produce the most suitable milk for production of cheese, with good rennet coagulation properties and high cheese yield.

In most goat breeds of Southern Europe, the frequency of weak and zero variants is low. Exceptions are the Spanish Canaria and Italian Garganica breeds, which have gene frequencies of 20% and 23%, respectively, for the zero variant (Caroli *et al*., 2007; Albenzio *et al*., 2009). It has also been shown that Norwegian dairy goats have a remarkably high frequency (70%) of the low or zero α_{s1}*-CN* allele (Vegarud *et al*., 1999; Devold *et al*., 2010). Since the Swedish Landrace is closely related to the Norwegian Landrace, it is likely that Swedish Landrace goats also carry the gene for zero synthesis of α_{s1}-CN.

2.8 Milk composition and cheese yield

Milk with a high concentration of fat and casein is required for cheese production, and these components have a major impact on both the quality and yield of the curd (Storry *et al*., 1983; Damian *et al*., 2008). Goat's milk containing higher amounts of fat, total protein and casein gives a higher cheese yield (Sanchez-Macias *et al*., 2010), coagulates more quickly and forms firmer curds (Clark & Sherbon, 2000). During coagulation, casein and calcium phosphate bridge micelles together to form a network which entraps fat and other solids (Walstra, 1999).

Any factor that influences milk composition also influences cheese making properties (Storry, 1983). Ruminant milk composition is influenced by breed (Soryal *et al*., 2005; Damian *et al*., 2008), age, stage of lactation, lactation number, month of sampling, nutrition and environmental, genetic factors, udder morphology, number of kids and suckling (Storry et al., 1983; Peris *et al*., 1997; Clark & Sherbon, 2000; Marnet & McKusick, 2001; McKusick *et al*., 2002a; Salama *et al*., 2003, 2004; Moatsou *et al*., 2004; Carnicella *et al*., 2008; Ahuya *et al*., 2009; Dønnem *et al*., 2011; Flores *et al*., 2011). It has also recently been demonstrated that grazing season and forage type influence goat's milk composition and rennet coagulation properties (Inglingstad *et al*., 2014). Milk composition in different goat breeds is illustrated in Table 2. Soryal *et al*. (2005) showed that milk from Nubian goats has higher fat, total protein and casein content than milk from Alpine goats, resulting in 2.7 and 1.7 kg of curd from 10 L milk, respectively. Milk obtained from the Swedish dairy goat has a lower dry matter content and thereby poorer coagulation properties, and it takes 10-12 L of milk to produce 1 kg of hard cheese (Lipage, pers. comm. 2010). Goats with a high solids content in the milk, particularly Nubians, are therefore recommended if cheese making is the objective, since coagulation rate and curd firmness are important economic factors (Clark & Sherbon, 2000).

Both the amount and the proportions of the caseins affect the cheese making properties. In particular, high amounts of α_{s1}-CN, but also κ-CN, have been shown to improve cheese making (Pirisi *et al*., 1994; Pierre *et al*., 1998; Clark & Sherbon, 2000). Furthermore, α_{s1}-CN has been shown to correlate positively with the total casein concentration in milk (Pirisi *et al*., 1994). Strong α_{s1}-CN milk is characterised by higher total protein, fat and calcium concentrations and by lower pH and smaller casein micelles than milk containing low or medium levels of α_{s1}-CN (Pirisi *et al*., 1994; Ambrosoli *et al*., 1988; Pierre *et al*., 1998; Clark & Sherbon, 2000). All these factors improve the cheese processing properties, such as rennet coagulation, cheese yield, gel firmness and quality (Ambrosoli *et al*., 1988; Clark & Sherbon, 2000; Devold *et al*., 2010). The coagulation time is generally shorter for caprine than for bovine milk (Walstra *et al*., 1999; Park *et al*., 2007), but the coagulum is less firm and gives lower curd yield (Pirisi *et al*., 1994). Moreover, strong α_{s1}-CN milk is reported to coagulate more quickly (Devold *et al*., 2010), although Ambrosoli *et al*. (1988) predicted that caprine milk with low α_{s1}-CN would coagulate more quickly than caprine milk with strong α_{s1}-CN.

Table 2. *Milk composition in different goat breeds.*

Country	Breed	Fat (%)	Protein (%)	Casein (%)	Lactose (%)
UK	British Saanen	3.5	2.6	2.3	4.3
UK	Nubian	4.9	3.6	3.5	4.5
France	Alpine/Saanen	3.6	3.2		
Italy	Sardinian	5.1	3.9		
Greece	Local	5.6	3.8	3.1	4.8
Cyprus	Damascus	4.3	3.8	3.0	
Spain	Murciano-Granadina		4.9	3.2	
US	Alpine	2.8	2.5	2.2	
Sweden	Swedish landrace	3.4	2.9	2.1	4.2
Norway	Norwegian landrace	3.4	2.9	2.1?	4.2

Information from different sources

2.9 Suckling and milk production

It has been debated whether milk production in animals kept in mixed milking-suckling systems is improved. Suggestions and results from earlier studies are contradictory, partly owing to comparisons being made between different breeds, species and milking regimes. It has been reported that milk obtained from ewes kept in mixed management systems contains a low fat concentration (McKusick *et al*., 2002a; Jaeggi *et al*., 2008; Tzamaloukas *et al*., 2015). Furthermore, Jaeggi *et al*. (2008) demonstrated that partially suckled ewes yielded milk with low fat levels, resulting in lower cheese yield (Table 3). McKusick *et al*. (2002) suggested that animals kept in mixed management systems have impaired milk ejection during machine milking, since the milk is only ejected when the offspring are present. Impaired milk ejection is reported to lead to lower milk yield and lower fat content, since milk fat synthesis may be inhibited and milk fat transfer from the alveoli to the cistern between milkings does not occur (Marnet & McKusick, 2002a). Impaired milk ejection in suckled animals can be restored to full function two weeks after separation from the offspring, when both milk yield and fat content are at similar levels as in dams milked only (McKusick *et al*., 2001; Mendoza *et al*., 2010).

In contrast, others have suggested that suckling plus frequent milking during early lactation improves mammary development by increasing both mammary proliferation and differentiation of mammary cells of goats (Wilde *et al.*, 1987). The mother-offspring bond has a very strong effect on regulation of the hormones involved in lactation, and interruption of this bond immediately after birth has been shown to result in negative effects on lactation persistency and less effective OT release during milking (Marnet & McKusick, 2001). OT-mediated milk ejection is necessary for complete emptying of the udder and also further stimulates milk secretion (Bruckmeier, 2005). Studies in ewes kept together with their lambs confirm that OT levels in plasma only increase during suckling and not during milking (Marnet & Negrao, 2000), who also showed that milk yield was higher in ewes during suckling compared with machine milking, and this has been confirmed by Delgado-Pertinez *et al.* (2009b).

Table 3. *Milk composition and actual cheese yield before brining from early ovine milks from two weaning systems. Mixed ewes are both suckled and machine milked for the first month of lactation. Ewes milked only are weaned from their lambs 24h postpartum.*

	Weaning System	
	Mixed	Milked only
Milk Fat (%)	**2.72**	**6.78**
Total Protein (%)	5.51	6.13
Casein (%)	4.32	4.75
Casein/Protein (%)	83	83
Total Solids (%)	14.24	18.51
Cheese Yield (%) →	**12.9 %**	**18.1 %**

Source: Modified version of a table by Jaeggi et al. (2008).

2.10 Measurements of suckling behaviour

There are difficulties in measuring total milk production during the suckling period (Cameron, 1998), since both milk off-take from milking and milk consumption by offspring must be considered. Different techniques have been used to estimate the milk yield, such as Weigh-Suckle-Weigh (WSW) (Delgado-Pertinez *et al.*, 2009a, 2009b; Hammadi *et al.*, 2012, Fernandez *et al.*, 2013), OT methods (Peris *et al.*, 1997; Fernandez *et al.*, 2013), milk off-take (machine or hand milking), isotope transfer from mother to young and isotope dilution (Shkolnik & Choshniak, 2006). The WSW and OT methods are considered to be the most appropriate for this purpose (Shkolnik & Choshniak, 2006; Fernandez *et al*., 2013).

Furthermore, different measurements of suckling behaviour have been used to estimate milk transfer from mother to offspring, for example suckle bout frequency and time spent suckling (Cameron, 1998). However, no single method seems sufficiently reliable to estimate total milk production (Cameron, 1998), and therefore a combination of different techniques is preferable.

2.11 Animal Welfare

Agricultural practices have changed considerably over the past century. The goal has been to increase production and efficiency and to satisfy consumer demand for animal products at low cost (Oltenacu & Algers, 2005). The low cost and high return model in the dairy sector today is based on reduction of feed costs by optimising use of resources, increased farm profit, reduced workload for farmers and reduced cost for consumers (Oltenacu & Algers, 2005; Marnet & Komara, 2008). The improved production efficiency has therefore led to genetic selection focused on increasing milk yield, but at the expense of reducing animal welfare. High yield in animals is often accompanied by increased incidence of health problems, declining ability to reproduce and declining longevity (Oltenacu & Algers, 2005). During recent decades there has been growing concern about the living conditions for farmed animals, and improving animal welfare at farm level has become one of the most debated issues in the field of animal husbandry (Battini *et al*., 2014).

The current debate on animal welfare often refers to modern animal production systems, where the animal's way of life has become progressively artificial (Miranda de la Lama & Mattiello, 2010). The opportunity for animals to express certain social behaviours can be limited by captivity and management systems, where goats often are reared intensively under high stocking densities. Dams and kids are exposed to early separation, frequent regrouping, sexual segregation and manipulation during critical periods, including gestation and weaning (Miranda de la Lama & Mattiello, 2010).

Dairy farmers, the dairy industry and consumers do not agree on the real impact when it comes to animal welfare and milk quality in modern management systems (Marnet & Komara, 2008), but there is also growing interest among dairy producers engaged in biological systems for a more respectful approach to animal behaviour and breeding on-farm (Marnet & Komara, 2008). One debated issue is early separation of mother and offspring, which is currently believed to be most profitable way of saving milk for human consumption (Miranda de la Lama & Mattiello, 2010). Both behavioural and productivity data indicate that early separation can be stressful for dam and kids (Boivin & Braadstad, 1996; Bergamasco *et al*., 2005; Napolitano *et al*., 2008; Newberry & Swanson, 2008; Miranda de la Lama & Mattiello, 2010; Winblad von Walter *et al*., 2010). Maintaining the mother-offspring relationship is likely to have positive consequences for the welfare of the mothers and their offspring (Hernandez *et al*., 2007). With respect to behavioural development and good animal welfare, Bungo *et al*. (1998) suggested that weaning should not occur before 6-7 weeks of age. Among feral goats, kids remain with their mothers from birth until 11-12 months of age (Miranda de la Lama & Mattielo, 2010). During the transition from maternal to artificial rearing, emotional and nutritional stressors in young animals have deleterious effects on their growth, health and vitality (Sevi *et al*., 2009). The animal welfare issue has partly reversed the trend for intensive management systems towards increased interest in more semi-intensive ways of keeping animals.

3 Aims

The overall aim in this thesis was to investigate if cheese processing properties of Swedish goat milk could be optimized by suckling and especially:

- To provide a random survey of the Swedish goat population with the aim to estimate the frequency of Swedish goats producing low levels of α_{s1}-CN (Paper I).

- To investigate how oxytocin and vasopressin is released during suckling compared with milking (Paper II).

- To investigate if vasopressin can cause milk ejection (Paper III).

- To investigate if the cheese-processing properties of goat milk could be optimised by appropriate management of suckled/milked goats (Paper IV).

Materials and Methods

This section gives an overview of the materials and methods used in the studies that are the basis of this thesis. Full descriptions are given in each paper (I-IV).

3.1 Animals and housing (Paper II-IV and all unpublished studies)

Swedish Landrace dairy goats (*Capra hircus*) were kept together with their kids in an indoor free-range stall with straw and shavings as bedding material at the Swedish University of Agricultural Sciences, Uppsala, Sweden. Hay, water and mineral licks were made available *ad libitum* and large plastic boxes and tables served as enrichment items (Figure 1). Room temperature was kept at 17±2 ºC. The care of the animals and the experimental procedures were approved by the Local Ethics Committee, Uppsala, Sweden.

3.2 Experimental procedures

Training and use of the goats was performed in accordance with the 3R (replacement, reduction, refinement) principles for more ethical use of animals in experimentation first described by Russell and Burch (1959). Refinement refers to methods that alleviate or minimise potential pain, suffering or distress and enhance the welfare of the animals used. Before the experiments, all animals were well adapted to handling and accustomed to the experimental procedures. When training each experimental procedure (shaving, local anaesthetic, insertion of catheter, milk and blood sampling *etc*.), the goats were offered concentrates and fruit as a reward. Milk and blood sampling and behavioural observations were performed by experienced researchers and technicians. To minimise any discomfort during blood sampling, local anaesthetic (Emla lidocaine ointment, AstraZeneca, Sweden) was applied on shaved skin over the jugular veins. Sixty minutes later, a catheter (Secalon T, Ohmeda, Swindon, UK) was inserted into the vein. In Papers II-IV, the experiments were performed in a cross-over design, thereby reducing the number of animals needed.

Figure 1. Goats and kids were kept together; tables and large boxes served as enrichment items.

3.2.1 Procedures before experiments

Before the experimental procedures in Papers II and III and other unpublished investigations, between 06.30-7.00 the goats walked to an adjacent room where they were milked upon arrival. The goats were temporarily held in individual cages, with free access to hay and water (Figure 2). Time schedule before and during each experiment is shown in Figure 3 (for Paper II) and Figure 4 (for Paper III).

Figure 2. Before the experiments started, the goats were trained and adapted to be kept in individual cages. After each experimental procedure (shaving, local anaesthetic, catheter insertion, milk and blood sampling *etc*.), the goats were offered concentrates and fruits as a reward (improvements of refinement in 3R).

3.2.2 Procedures during experiments in Paper II

At 10 and 5 min before suckling or milking (time zero), blood samples 1 and 2 were taken as controls. The udder was then massaged and samples 3 and 4 were drawn after 0.5 and 1.0 min, respectively (Figure 3), whereupon milking started and the following blood samples 5-12 were taken in immediate sequence (Figure 3). The milker and the person taking blood samples synchronised their timing so that the speed of blood removal was adjusted to the rate of milking. In this manner, the samples were evenly distributed during the milking and the samples could be compared between goats. Samples 13-16 were taken after milking ended in the animal. The sampling procedure was in principle the same in the suckling treatment, except that the goats were sniffing their kids through the box wall when samples 3-4 were withdrawn and were suckling when samples 5-12 were taken. The kids suckled for about the same time as it took to milk their mother. The kids stayed with their mother during the rest of the observation period.

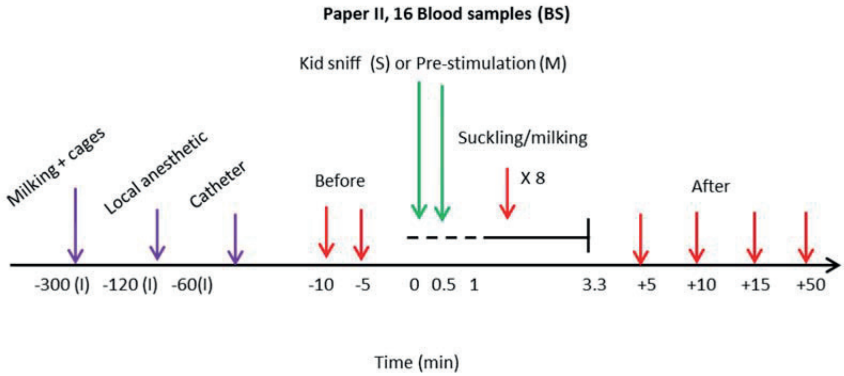

Figure 3. Goats walked to individual cages where they were milked upon arrival. Local anaesthetic was applied on shaved skin over the jugular vein and a catheter was inserted 1 h later. At 10 and 5 min before suckling or milking, blood samples 1 and 2 (red arrows) were taken as controls. At time 0-0.5 min, the udder was pre-stimulated before milking or dams sniffed their kids before the suckling treatment. During milking/suckling, eight blood samples (5-12) were collected. After milking/suckling has stopped, a further four blood samples (13-16) were collected after 5, 10, 15 and 50 min.

3.2.3 Procedures during experiments in Paper III

The procedures used in the experiments in Paper III are outlined in Figure 4. Three hours after morning milking, the experiment started with collecting the first blood sample (Bs1, Figure 4), followed by cisternal milking (CM, one teat only). Thereafter, Bs2 was taken, followed by an OT (5IU) or AVP (0.15 IU) injection (needle and syringe) in the contralateral vein. At 3 min after cisternal milking, alveolar milk was emptied and the experiment finished with withdrawal of Bs3.

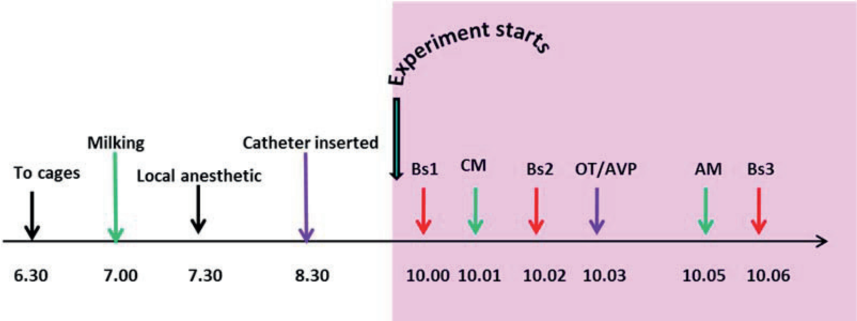

Figure 4. Goats walked to individual cages where they were milked upon arrival. Local anaesthetic was applied on shaved skin over the jugular vein and a catheter was inserted 1h later. The experiment started 3 h after the morning milking with collection of the first blood sample (Bs1), followed by cisternal milking (CM). Blood sample 2 (Bs2) was then collected, followed by OT (OT) or AVP (AVP) injection. Two min after injection, the alveolar milk was emptied and finally the last blood sample was collected (Bs3).

3.2.4 Experimental procedures in Paper IV

Before experiments, dams were kept together with one kid for 24 h, but during the four experimental weeks, dams were partially kept together with their kids. Four treatments were applied; two milk accumulation intervals (Short, Long) and two milking regimes (Suckling Before Milking = S; Milking Before Suckling = M). By definition, milk accumulating interval is referred to the separation period between dams and kids. In the Short accumulation treatments, dams and kids were kept together for 16 h (T16) at night; in T16-S dams were suckled before milking (S-M) and in T16-M dams were milked before suckling (M-S). In the Long accumulation treatments, dams and kids were kept together for 8 h (T8) in daytime; in T8-S dams were suckled before milking (S-M) and in T8-M dams were milked before suckling (M-S). In the Short accumulation treatments, dams were milked twice daily (M and S-M or M-S) and in the Long accumulation treatment dams were milked once daily (Figure 1 in Paper IV). Each treatment lasted for one week (Saturday to Friday), with five sampling days per week (Monday-Friday). The study had a cross-over design and all goats received all treatments. During the first two days of each treatment (Saturday-Sunday), no data were collected.

3.2.5 Procedures used in unpublished studies

Milk composition was determined in an unpublished study where dams and one kid were kept together exclusively for 24 h per day from day 5 until day 70. If a dam had twins, one of the kids was removed and raised artificially from day 4 of lactation. Kids that remained with their dams were only allowed to suckle one teat, while the other was covered with a bra suspended in harness to prevent suckling (Figure 5). Dams and kids stayed in their home pen (see Animals and housing) all day, except during the milking procedures.

Figure 5. Kids were only allowed to suckle one teat, while the other was covered with a bra suspended in harness to prevent suckling.

3.3 Hormone analyses

For hormone analyses, samples of blood were taken from the jugular vein via the catheter and a syringe and transferred to ice-chilled K3-EDTA tubes (Sarstedt, Nümbrecht, Germany). The tubes were centrifuged at 1500 *g* at 4 °C for 20 min and stored at -80 °C until assayed.

All assays were validated for caprine plasma by showing that dilutions of the plasma were parallel to the standard curve. AVP were analysed by radioimmunoassay (Coat-A-Count, DPC, Los Angeles CA, USA), as previously described (Hydbring *et al.*, 1999). OT was analysed using an OT ELISA kit for OT (Electra-Box Diagnostica AB, Tyresö, Sweden) and evaluated for goat plasma in our laboratory (Norrby *et al.*, 2011).

3.4 Estimation of suckled milk volume (Paper IV)

In order to estimate total milk production, a method was developed to estimate the suckled milk volume. This was done by a Weigh-Suckle-Weigh (WSW) method in combination with observation of suckling behaviour during the free suckling periods.

3.4.1 Weigh-Suckle-Weigh

During Weigh-Suckle-Weigh (WSW), the suckled milk yield and milk flow were estimated. First, the kids were weighed and then allowed to suckle to satiation. The time recording started when the kid had the teat in its mouth and the tail flicked and stopped when the kid left the teat (or the dam went away). Finally, the kids were weighed again and both suckling duration (s) and weight gain of each kid (g) were recorded. The milk flow (g/s) was calculated by dividing kid body weight increase (g) by the time taken to suckle that amount (s).

3.4.2 Behavioural observations

In Paper IV, suckling behaviour was observed during four days in total, two days in T16 (T16-S and T16-M) and two days in T8 (T8-S and T8-M). Six kids were continuously observed during 16 h of free suckling at night (T16-S and T16-M) and the other six kids for 8 h during daytime (T8-S and T8-M). Each time a kid suckled this was recorded and the duration of each bout was clocked in seconds. If several kids started to suckle at the same time, three stopwatches were used for recording (two observers during the most frequent suckling periods). The time recording started when the kid had the teat in the mouth and the tail flicked and stopped when the kid left the teat (or the dam went away). In addition, disrupted suckling sessions (disrupted by dams, kids or other goats), choice of teat (if kids preferred to suckle both, left or right teats) and suckle attempts (if the dam rejected suckling by the kids) were recorded for each suckling session. These behaviours are presented as percentage of total observations. Total milk yield (24 hours) was determined as the sum of: milk off-take from milking (milk yield available for processing), estimated milk yield from WSW and suckled milk yield estimated from behavioural observations: Total milk yield (g) = Milk off-take + WSW + Suckled milk yield.

3.5 Milking procedures

The goats were machine milked twice daily at 07:30 in the morning (MM) and 15:30 in the afternoon (AM) for 10 weeks (unpublished results), or milked once (1X) or twice (2X) daily in Paper IV. One goat was milked at a time and a mixture of feed concentrate (Edel komet, Uppsala, Sweden) and 50% oat (Wollerts, Uppsala, Sweden) and carrots was offered in connection with each milking. Machine milking was performed at a vacuum pressure of 42 kPa with a pulsation ratio of 90 pulses/min (De Laval International AB, Tumba, Sweden). In unpublished studies, both udder halves were milked separately with a special separate-milker 8L-bucket machine (provided by DeLaval International AB, Tumba, Sweden). In all other studies, the dams were hand milked twice daily in the cages.

3.6 Milk Composition

In Papers II-IV and unpublished studies, milk samples were collected, weighed (Mettler Toledo, 98 Stockholm, Sweden) and analysed directly after each milking. For analyses, the samples were thoroughly mixed and 10 mL of milk were pipetted into plastic tubes. The tubes were heated in a water-bath to 40 ºC and analysed for fat, total protein and lactose concentration using a mid-infrared spectroscopy (MIR) method (Miris AB, Uppsala, Sweden).

3.6.1 Somatic cell count (SCC)

Somatic cell count (SCC) was measured in fresh milk by fluoro-opto-electronic cell counting (Fossomatic 5000, Foss Electric, Hillerød, Denmark). The milk pH was measured by a pH meter calibrated for goat's milk (FiveGo^{TM} pH, Mettler Toledo, Stockholm, Sweden) in fresh milk directly after milking.

3.7 Casein and individual curd yield

Prior to studies by Högberg *et al*. (2011a, 2011b), the casein concentration in Swedish Landrace goats was unknown. In this thesis, the methodology used in those studies for analysing the casein concentration was refined, both by testing different methods and by developing a method for measuring the individual curd yield in Swedish dairy goats. The methods were designed to fit both laboratory and on-farm analyses.

3.7.1 Measuring casein concentration and individual curd yield by a centrifugation method

The casein concentration was measured in Paper IV and unpublished studies, while the individual curd yield was only measured in Paper IV, using a method inspired by Othmane *et al*. (2002). For analyses of both casein concentration and individual curd yield, a rennet-coagulation method was used as described in Figure 6. Total protein from the initial milk samples minus whey protein obtained by the MIR method gave the casein concentration.

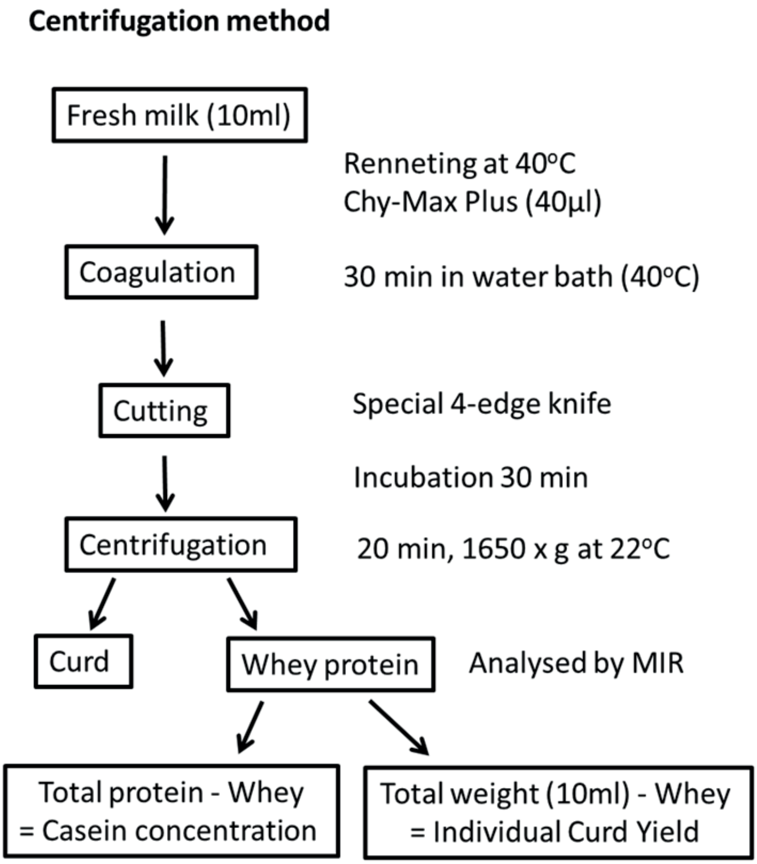

Figure 6. Chy-Max was added to 10 ml of fresh milk (40°C) and vortexed. The samples were set to coagulate for 30 min before the curd was vertically cut into four equally sized sections with a four-edged knife specially made to fit the tubes. After another 30 min of incubation at 40 ^{o}C, the samples were centrifuged at 1650 x g for 20 min at 22 °C. The whey was expelled from the curd and weighed in tared tubes. Curd yield was determined from the weight difference between the initial milk sample and the expelled whey and expressed as grams of curd per 100 g milk and presented as a percentage.

The methodology for measuring the individual curd yield by centrifugation proved unsuitable for samples with a high fat concentration (>5%). In milk samples with a high fat concentration (>5%) the curd split and one part of the curd stayed in the upper part of tube (Figure 7, right). Therefore these samples were excluded from the analysis of individual curd yield, but were still used when measuring the whey fraction and calculating the casein concentration. All milk samples with a fat concentration below 5% were included in all analyses.

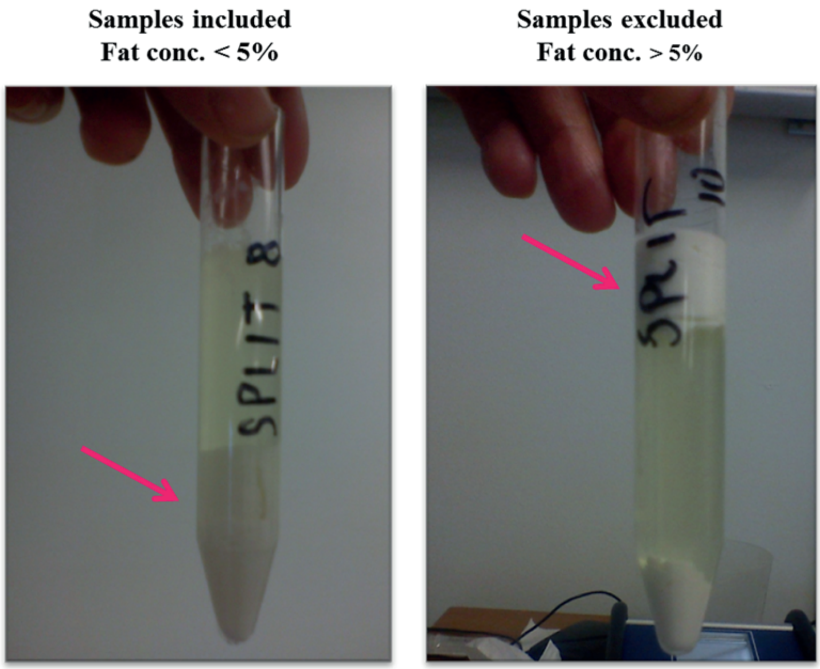

Figure 7. The method for analysing the individual curd yield was unsuitable in samples with a high fat concentration (> 5%). In samples with a fat concentration below 5%, the curd was found in the bottom of the tube (left). In samples with a fat concentration above 5% the curd split and part of the curd floated up and stayed in the upper part of the tube (right). These latter samples were excluded from the analysis of individual curd yield, but were still used when measuring the whey fraction and calculating the casein concentration.

3.7.2 Measuring casein concentration by a filtration method

It can be problematic to measure the casein concentration on farms using a centrifugation method. Therefore, a filtration method used previously on farms (Figure 8, left) was tested for reliability by comparing the casein concentration from the filtration method with the casein concentration measured from the centrifugation method (dual sub-samples from the same milk sample, n = 35 x 2). In the filtration method, 60 µL of $CaCl_2$ ($CaCl_2$ 48% = 80 g $CaCl_2$ + 100 mL H_2O) were added to 20 mL fresh milk. Thereafter, 200 µL standard rennet (75% chymosin and 25% pepsin, Kemikalia AB, Skurup, Sweden) were added to the tubes during gentle stirring. The samples were set to coagulate for 5 min and then cut vertically into nine pieces and post-heated for another 5 min. The curd was filtered from the whey and poured into 10 mL plastic tubes containing one drop of a special detergent (Triton X®-100, Merck KGaA, Darmstadt, Germany).

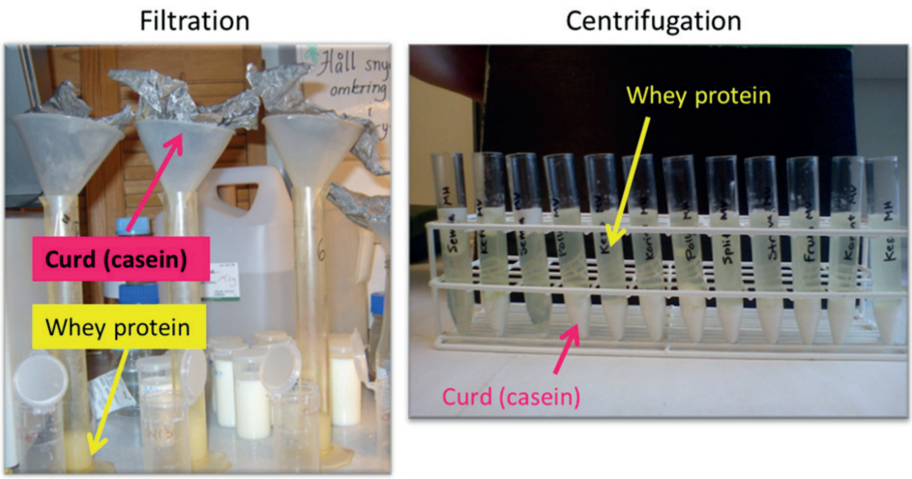

Figure 8. Two different rennet coagulation methods: filtration (left) and centrifugation (right), were tested for reliability in analysing the casein concentration in milk.

3.8 Animals and experimental procedures in Paper I

Milk samples were collected from 283 Swedish dairy goats on 28 farms in 10 different geographical regions of Sweden. All farms produced their own goat's cheese on-farm.

3.8.1 Protein profile (Paper I)

A sample of 5 mL milk was collected from each goat and the protein profile was analysed by capillary zone electrophoresis (CZE). Before analysis, the samples were defatted at 3000 x g at 4 ºC for 10 min and protein separation was performed according to Åkerstedt *et al.* (2012). The goat's milk proteins were identified by comparison with reference samples and calculation of relative concentrations of the individual proteins was based on the peak area and expressed as a percentage of the total area recorded for all peaks in the electropherogram (Figure 9). The percentage of α_{S1}-CN compared with the other caseins was calculated and used to classify goats as strong (15-25%), medium (7-14.9%) and low (0-6.9%) producers of α_{S1}-CN.

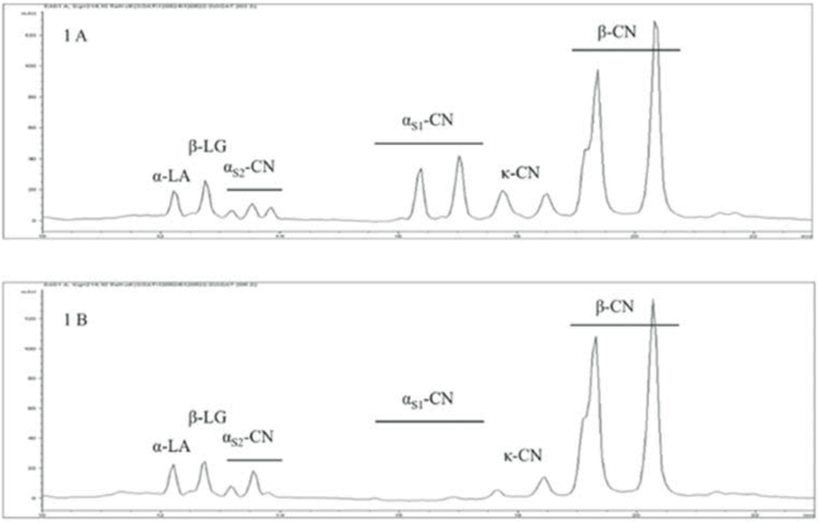

Figure 9. Representative electropherograms of the protein profile in goat's milk. 1A) Protein profile with high expression of α_{S1}-CN and 1B) protein profile with low expression of α_{S1}-CN. The individual proteins are indicated. α-LA, α-lactalbumin; β-LG, β-lactoglobulin; α_{S1} CN, α_{S1}-casein; α_{S2}-CN, α_{S2}-casein; κ-CN, κ-casein; β-CN, β-casein.

4 Results and Discussion

This chapter presents a summary of the results from Papers I-IV, together with some preliminary results from unpublished studies. More detailed information can be found in each individual paper.

Before this work, information about the genetic protein profile of goat's milk, particularly the α_{S1}-CN concentration in milk from Swedish dairy goats, was lacking. The first step was therefore to investigate the cheese making properties of Swedish goat's milk by analysing the genetic protein profile (*α_{S1}-CN*) in 283 Swedish dairy goats.

The next step was to investigate whether the milk fat concentration could be improved by suckling. Before this work, knowledge about how suckling influences milk production in the Swedish Landrace dairy goat was lacking. The results presented in this thesis mainly focus on how suckling and milking influence the milk ejection reflex in the Swedish Landrace goat and also how suckling influences the cheese making properties of goat's milk.

The studies demonstrated how hormone levels in plasma, milk yield, milk composition, curd yield and SCC were affected when dams and kids were kept together in mixed management systems. The kids' milk consumption was also taken into consideration by observing the suckling behaviour during the free suckling periods. In Paper I, the genetic protein profile was determined in milk from goats in Swedish dairy herds.

4.1 Protein profile in Swedish dairy goats

Poor cheese processing properties have been observed in milk from Swedish dairy goats and one possible underlying factor was investigated in Paper I. Analysis of the milk samples collected from different dairy goat herds revealed a high frequency of samples from Swedish dairy goats with low expression of α_{S1}-CN. Of 283 goat milk samples, 185 (65%) had low expression of α_{S1}-CN (below 7% concentration). Only 35 goats (12%) produced high concentrations of αS1-CN, while 63 (22%) were considered medium producers (Figure 10, upper left). The goats from the herd at SLU showed the second best expression of α_{S1}-CN among farms in Sweden (Figure 10, upper right), with 36% showing high and 36% medium expression of α_{S1}-CN. In five of the herds investigated, 100% of the goats had low α_{S1}-CN expression (Figure 10, lower left), and the herd with the highest expression of α_{S1}-CN showed no expression of the low variant (Figure 10, lower right). There was no relationship between the different casein profiles and regions (Paper I).

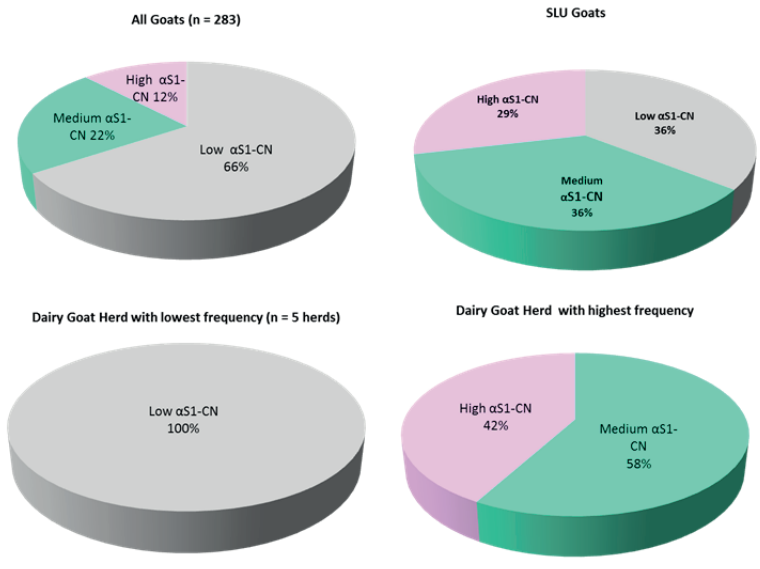

Figure 10. Proportion of Swedish dairy goats expressing high, medium or low levels of α_{S1}-CN. All goats (upper left) represents the total number of goats included in the study. Only 36% of the goats from the goat herd at SLU (upper right) expressed low levels of α_{S1}-CN. The farm with the lowest frequency had 100% low α_{S1}-CN expressers (lower left) and that with highest frequency (right) 0% low α_{S1}-CN expressers.

The high frequency of Swedish dairy goats expressing the low variant of α_{S1}-CN in milk was actually not surprising, as the Swedish Landrace is closely related to the Norwegian Landrace, which has been shown to produce low levels of α_{S1}-CN in milk and with up to 70% of the Norwegian goat population characterised by low expression of α_{S1}-CN (Devold *et al*., 2010; Dagnachew *et al*., 2011). Lack of this protein is connected to poorer coagulation properties and lower cheese yield (Clark & Sherbon, 2000; Johansson *et al*., 2014). Milk with a higher proportion of α_{S1}-CN has improved cheese making properties due to greater total protein, fat and calcium contents (Pierre *et al*., 1998; Clark & Sherbon, 2000).

The reason for the high frequency of the zero variant of α_{S1}-CN expression in Norwegian goats is believed to be associated with brown cheese production (whey cheese), which was historically the main Norwegian goat's milk product. When manufacturing this type of cheese, the dry matter content in milk is of less importance because the whey is the main component and not the curd. Swedish dairy goats are believed to yield more than Norwegian goats and are among the highest yielding goat breed in the world, which could also contribute to the low dry matter content in milk. However, there is a need for better characterisation of the Swedish goat population. The unfavourable mutations of the α_{S1}-*CN* gene result in negative economic consequences for goat's cheese production.

On the other hand, it is important to consider the low number of animals existing in Sweden today, with only about 14 000 goats of the Swedish Landrace breed registered. Since the breed is endangered and maintenance plans are in place for its preservation, it is important to be careful when breeding goats with strong milk aimed for cheese processing. Therefore, other ways of improving the cheese processing properties of goat's milk must be considered, *e.g*. improving the milk fat concentration. In the remainder of this thesis, different suckling strategies to improve the milk fat concentration in goat's milk are described.

From another point of view, the low concentration of α_{s1}-CN may have beneficial effects for human digestion (Park *et al*., 2007). As human milk lacks α_{S1}-CN, the low levels of α_{S1}-CN in some goat's milk and the higher proportion of β-CN mean that the casein profile is closer to that of human milk than is the casein profile of cow's milk (Silanikove *et al*., 2010). It is also common for some people allergic to cow's milk to be able to drink goat's milk. The real reason why people who are allergic to cow's milk can consume goat's milk is suggested to be the different epitopes in goat's milk. Studies have shown that milk from goats that produce lower levels of α_{S1}-CN is potentially less allergenic than milk from goats with higher levels of this protein (Bevilacqua *et al*., 2001; Ballabio *et al*., 2011; Hodgkinson *et al*., 2012). However, it is unfortunately often believed that people suffering from lactose intolerance are able to consume goat's milk products, which of course is wrong, although cheese and fermented products contain no or very low levels of lactose. Even though goat's milk contain less lactose than cow's milk (on average 4.1% compared with 4.7%), it is not a dietary solution for people suffering from lactose intolerance (Silanikove, 2010).

4.2 Hormone levels in plasma during suckling and milking

Another reason for the low milk fat concentration in milk from Swedish dairy goats might be impaired milk ejection during milking. The second step in the present thesis (Paper II) was therefore to investigate whether the physiological milk ejection reflex differs during suckling compared with milking, by measuring plasma levels of oxytocin (OT). Paper II also investigated whether vasopressin (AVP) plays a role in the milk ejection reflex.

The results in Paper II demonstrated that plasma levels of both OT and AVP increased during suckling, but not during hand milking ($P<0.05$). These results imply that AVP and OT release is stimulated by the kids. The parallel change observed in these two hormones during suckling supports the suggestion that AVP increases due to the same stimulus as OT. None of the peptides increased while the goats could hear, see and smell their kids (Figure 11), confirming findings by Hernandez *et al*. (2002). When the kids started to suckle, the plasma OT level rose abruptly and remained elevated until the kids stopped suckling ($P<0.05$).

However, hand milking was not accompanied by increased OT concentration. The lack of OT release can probably be explained by maternal behaviour and lack of a proper signal normally present during suckling of their own offspring (Marnet & Negrao, 2000; Hernandez *et al*., 2002). It is known that goats establish a selective bond with their kids post-partum. In the present study, all goats and their kids were kept together in a large pen between experiments and bonding between goats and their kids was well established.

On the other hand, it has been shown that plasma OT concentration does not always increase during milking in goats (McNeilly, 1972; Mosdøl *et al*., 1981), even if dams are milked only (no suckling). One explanation could be that the mammary glands in goats differ anatomically and physiologically from those in cows, since goats are able to store large amounts of milk in their large cisternal glands (up to 80%) between milkings. That portion of milk can be drained without OT release (Mosdøl *et al*., 1981; Marnet & Negrao, 2000), which explains why the milk ejection reflex is less important for milk removal in goats (Peris *et al*., 1997; Marnet & McKusick, 2001), and why OT is not positively correlated with milk flow emission patterns (Bruckmaier *et al*., 1994a), since cisternal milk is available for a long period without interruption of milk flow (Bruckmaier & Blum, 1996). This contradicts findings for dairy cattle (Mayer *et al*., 1984), which in contrast to small ruminants store only 20% of the milk in the cisternal fraction and, unlike goats, are dependent on milk ejection for milk availability.

The impaired milk ejection during the cisternal milkings could also be explained by the management system before the experimental periods. The goats in Paper II were kept in mixed systems (milking combined with suckling) and they were not adapted to regular machine milking procedures in an early stage of lactation. These types of management systems can cause failure of milk ejection, McKusick et al. (2002a) showed that when ewes and lambs were kept together, only cisternal milk was obtained during milking.

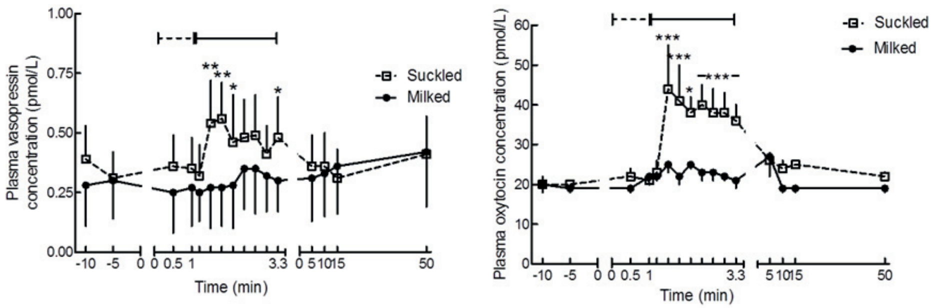

Figure 11. Plasma AVP (left) and plasma OT (right) concentration during suckling and hand milking. Blood samples were taken 10 and 5 min before, and at 0.5 and 1 min during pre-stimulation or while the goat could sniff at her kids (|-----|) Eight blood samples were taken as evenly as possible while the goats were milked or suckled (|——|). Samples were taken 5, 10, 15 and 50 min after end of milking and suckling. Values are means ± SEM. **P* < 0.05 and ***P* < 0.01 suckling *v*. hand milking.

4.3 Vasopressin and milk ejection (Paper III)

In Paper II it was demonstrated that suckling caused elevated plasma levels of both OT and AVP, which raised further questions about the function of AVP in connection with mammary function and milk synthesis during suckling or milking. The two neuropeptides are closely related and are able to bind on each other's receptors, although with less affinity (Zingg, 1996; Akerlund *et al*., 1999), which suggested that AVP might act on the OT receptors on the myoepithelial cells in the mammary gland. The next step in the thesis was therefore to investigate if AVP could cause milk ejection similar to that caused by OT (Paper III).

The results demonstrated that the alveolar milk yield increased after both AVP and OT administration compared with control treatments (no injection or saline; Figure 13, left). The proportion of alveolar milk to total milk volume was 15% and 21% (saline) in control conditions, 57% after OT and 56% after AVP administration. The cisternal milk yield was similar in all treatments (Figure 13, left).

The milk fat concentration increased after both OT and AVP administration (Figure 12, right), which clearly demonstrates that the fat-rich milk stored in the alveolar region was released into the cisternal fraction after treatments. For the first time, it was shown that AVP can cause milk ejection in the lactating goat, a finding which was further supported by the unchanged plasma levels of OT during the experiments (Figure 12, right). The cisternal milk had a low fat concentration before both OT and AVP treatment. There was no alteration in OT during and after the cisternal milking, which is a strong indication that only cisternal milk was obtained during these milkings (Cross, 1977). Thus hand milking without peptide injections in the mixed management system did not stimulate the release of OT. Two minutes after OT and AVP administration, both peptides increased above basement levels (OT: 1039 pmol/L; AVP: 26 pmol/L).

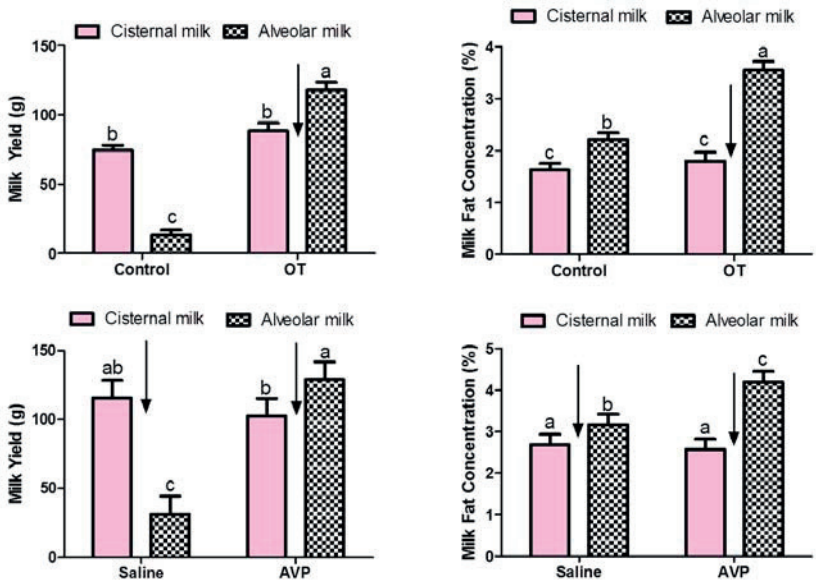

Figure 12. Milk volume (left) and fat concentration (right) from one teat each when eight goats were hand milked during two sessions. First, the cisternal milk (CM, open bars) was emptied and directly thereafter vasopressin (AVP), oxytocin (OT) or saline (0.9% NaCl) was injected into the jugular vein (arrows). Two minutes after injection, the alveolar milk (AM, hatched bars) was emptied. During control milkings, no injection was given. Values are presented as Least Square Means (LSM) ± standard error of mean (SEM). Means within the same series with different superscript letters (a-c) differ ($P<0.01$ or $P<0.001$).

Three hours after OT and AVP administration, milk composition was analysed again and some differences were observed in milk protein concentration, suggesting that although OT and AVP both cause milk ejection, their action is slightly different (data not shown). There was an increase in the milk protein content 3 hours after OT treatment, but not after AVP injection.

These results are in agreement with a study by Lollivier *et al*. (2006) in which *in vitro* addition of OT to rat mammary gland increased α_{s1}-CN accumulation near the apical membrane 1 min after injections, but no localisation of α_{s1}-CN was detectable after AVP injection. OT stimulates *in vitro* intracellular transport of newly synthesised caseins from the rough endoplasmic reticulum to the golgi apparatus and secretory vesicles in lactating rabbit mammary fragments (Olliver-Bousquet, 1976).

In addition, Dahlborn *et al*. (1990) showed that protein synthesis recovery is delayed after rehydration in severely dehydrated goats, even after milk volume is restored.

As regards the reasons for AVP release, Suzuki *et al*. (2000) found that both plasma OT and AVP increased during suckling in the rat and suggested that the role of AVP is to save water for milk production. This is also a plausible explanation for the suckling-induced AVP release in the goat (Paper II). Since milk mainly consists of water, it could be beneficial to increase water absorption in the kidneys when new milk is to be synthesised. Another explanation could be that the release of large amounts of AVP in severely dehydrated goats (Dahlborn *et al*., 1990; Olsson *et al*., 1996; Mengistu *et al*., 2007) acts on the myoepithelial cells and transfers the fat-rich alveolar milk to the cistern and makes it available to the offspring.

4.4 Acute and long-term effects of suckling

4.4.1 Acute-term effects of suckling

In Paper II it was demonstrated that suckling caused elevated OT and AVP levels in plasma, while in Paper III it was demonstrated that fat-rich milk stored in the alveolar region was released into the cistern after injections of OT and AVP. These results show the importance of measuring both OT in plasma and milk fat concentration when studying effects of milk ejection. In addition to Paper II, a second series (unpublished) was therefore added, where both milk fat concentration and plasma OT were measured simultaneously during suckling (day 1) and during milking (day 2). The dams were kept in a management system based on both suckling and milking (mixed management), as the aim was also to study the acute effects on milk fat concentration of these types of management.

The results showed that both plasma OT (Figure 13, left) and milk fat concentration (Figure 13, right) increased significantly during suckling, but not during hand milking in goats. This clearly demonstrates that suckling improves the neuroendocrine milk ejection reflex (Marnet & Negrao, 2000; Paper I) by releasing fat-rich alveolar milk into the cistern (Paper III). During suckling, the fat concentration increased by 50% in the milked teat and by 64% in the suckled teat. During milking, the fat concentration increased by 25% in both the milked and suckled teats.

The reason for the low fat concentration during milking is probably that milk ejection during milking is impaired and milk fat transfer from the alveoli to the cistern between milkings does not occur (McKusick *et al*., 2002a). Impaired or inhibited milk ejection can also be caused by milking in unfamiliar surroundings or if the animals are unaccustomed to the milking procedures (Bruckmaier, 2005). This was probably not the case in the present experiments, since the dams were well adapted to the experimental procedures, as verified by their plasma cortisol concentrations remaining at basement levels during the entire experiment (data not shown).

However, plasma OT levels were not increased in the hand-milked goats. Similar as in Paper II, and similar as in earlier findings in cows, where suckling caused higher oxytocin levels than milking (Lupoli *et al*., 2001). We suggest therefore that suckling causes a stronger and more complex sensory input when dams are kept in mixed management systems.

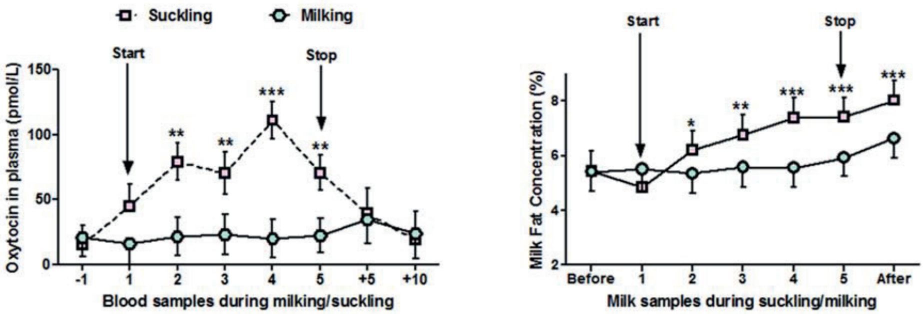

Figure 13. OT in plasma (left) and milk fat concentration (right) during suckling (squares) compared to milking (circles).

4.4.2 Long Term Effects of suckling

Milk from Swedish dairy goats separated from their kids generally contains a fat concentration, around 3.0 - 3.5% (Sjödin, 1979; Högberg *et al*., 2011b), which can be explained by different factors, such as genetics, housing, milking managements, feeding, handling and so on. It could also be explained by insufficient milk ejection during milking, in response to irregular OT and release during milking. After showing that the milk fat concentration increased more during suckling than milking in an acute situation a second series of studies (unpublished) was added to investigate the long-term effects of suckling and milking on milk fat concentration. For this, dams were kept together with one kid each and were milked twice daily for 10 weeks. The kids were only allowed to suckle one teat, while the other was covered with a bra (Figure 6) to prevent the kids from suckling.

The results showed that there was no difference in milk fat concentration between the milked and suckled teat in total (per day), even if the milk volume was lower in the suckled teat compared with the milked teat (Figure 14, left). At morning milking, the fat concentration was higher in the suckled than the milked teat, but at afternoon milking, the fat concentration was higher ($P<0.05$) in the milked than in the suckled teat (Figure 14, right).

However, there were great individual differences between animals in both milk yield and fat concentration. Even when the dams were kept together with one kid for 24 h, some individuals yielded 3-4 kg milk daily, containing a fat concentration of 4-5%. Individual differences in milk yield, both within and between treatments and individuals, seemed to be connected to udder size, parity and size of the animal. It has previously been shown that udder morphology and milk yield is positively correlated (Marnet & McKusick, 2001; Salama *et al*., 2004; Capote *et al*., 2006). The individual variation between goats thus shows that high yielding goats (high milk off-take) are more appropriate for mixed management systems than low yielding ones, some goats are able to produce about 10 kg of milk per day (Haenelin, 2007).

It is possible that more frequent udder stimulation could stimulate local synthesis of milk fat within the mammary gland (Knight & Wilde, 1993) or that more frequent milk ejection increases the proportion of fat-rich alveolar milk, which ultimately increases the total milk fat concentration (Knight & Dewhurst, 1994). Since there was no difference in milk fat concentration between the suckled and milked teats, it can be suggested that Swedish dairy goats have large cisterns to store large amounts of fat-rich alveolar milk once ejected by suckling. This milk could thereby be available for cheese processing, since the kids not are able to empty all milk (especially high producing animals; Marnet & Komara, 2008). It might thou be less efficient to have animals with small cisterns in mixed managements, as for example, dairy cows, that only are able to store about 20 % in their small cisternal glands (Marnet & McKusick, 2008; Mendoza *et al*., 2010). More frequent suckling or milking could also enhance milk synthesis in goats with large cisterns and lead to smaller amounts of milk accumulating in the alveoli (Marnet & Komara, 2008), which otherwise inhibits milk synthesis (Wilde *et al*., 1987; Li *et al*., 1999; Fitzgerald *et al*., 2007).

Another factor that might be important when goats are reared in mixed regimes is when the adaptation to milking occurs. The successful results in this study (both high fat content and high milk yield) could also be due to the goats being adapted to machine milking in an early stage of lactation.

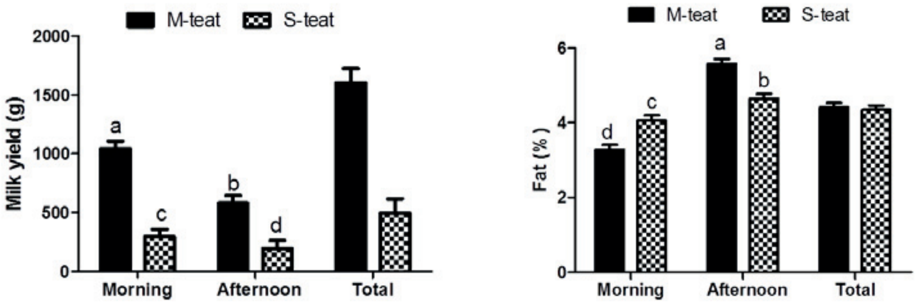

Figure 14. Long-term effects on milk yield (left) and fat concentration (right) during 10 weeks when one teat was machine milked twice daily, while the other teat was suckled by one kid. The milked teat was covered with a bra suspended in harness to prevent suckling.

4.5 Mixed management systems and suckling behaviour

An important aspect of animal production is to develop systems in which the animals can express their natural behaviour. The opportunity to express natural behaviour, *e.g.* by rearing dams and kids together for a longer period, improves well-being and animal welfare. Suckling is considered a positive situation, whereas abrupt and early separation of mother and offspring may have negative consequences for the welfare of the animals (Newberry & Swanson, 2008). Maintaining the mother-offspring relationship is likely to have positive consequences for the welfare of the mothers and their young (Hernandez *et al*., 2007). However, early separation of dams and kids is believed to be the most profitable way of saving milk for commercial purposes. One way to save more milk for the dairy if mother and offspring are kept together is to apply a partial suckling regime. It has been shown that these types of regimes can influence milk production positively in dairy sheep (Knight *et al*., 1993; McKusick *et al*., 2001, 2002a; Rassu *et al*., 2015).

In Paper IV, dams and kids were kept together in different mixed regimes. In one part of Paper IV, the aim was to investigate how different management systems affected the suckling behaviour and the kids' milk consumption. Kids were allowed to suckle freely for part of the time, either 16 h at night or 8 h in daytime. The suckling behaviour was observed to estimate the suckled milk yield during the free suckling periods, and a WSW method was used to calculate the milk flow (g/s). The number of suckling bouts and their duration were recorded during the free suckling period for 16 h (T16) and 8 h (T8). The total milk production was finally estimated by duration (s) x g/s and is presented in section 4.6.

4.5.1 Influence of separation time on milk consumption

Separation time affected the kids' milk consumption during weigh-suckle-weigh (WSW) measurements. In the longer separation treatments (16 h = T8), the suckling duration was longer (51 ± 6s) than with shorter separation (8 h = T16) (40 ± 6s, $P<0.05$), as illustrated in Figure 15 (left). In T8 the kids also consumed more milk (509 ± 60 g) than in T16 (405 ± 60g, Figure 15, right, $P<0.05$). These results imply that the kids were affected by the different management regimes, as they were more eager to suckle when they had been separated for a longer time. The suckling behaviour during WSW measurements was similar between days, although milk intake tended to decrease with time spent in the treatment. In the T8 treatment, the highest milk intake was observed on day 2, which is an interesting observation. It appeared that the kids habituated to the treatment and maybe learned that that they would be reunited with their dam after the separation periods. The kids consumed more milk in the T8 treatment, compared with T16, but there was no difference in milk flow between the treatments. (9.3 ± 0.7 g/s for T16-S, 8.9 ± 0.6 g/s for T8-S). These results imply that it is important to consider that the separation affects the kids' milk intake when using WSW. When estimating the suckled milk intake by WSW, milk flow has to be used in addition to behaviour observations during normal suckling conditions. The individual variation was large between animals, with one kid consuming more than 1 kg of milk within a few minutes, whereas others drank lower volumes.

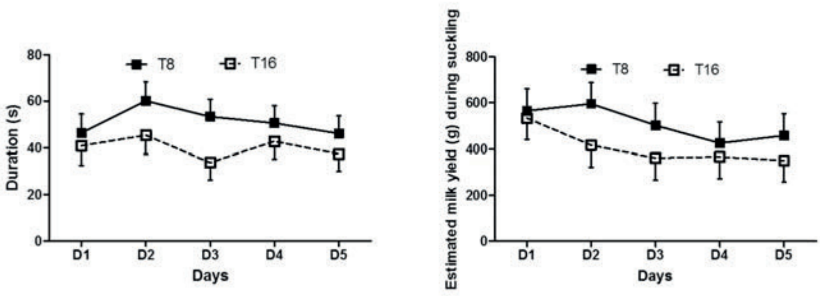

Figure 15. Duration of each suckling clocked in seconds during Weigh-Suckle-Weigh (WSW), (left). The weight gain after suckling was used to determine the kids' milk consumption (right). The milk flow was calculated as weight gain (g)/duration of each suckling (s). Values are presented as least square means ± standard error of mean (SEM).

4.5.2 Suckling behaviour during 16 and 8h of free suckling

During the free suckling periods, the number of suckling bouts and their duration were observed when dams and kids were kept together for 16 h (T16) or 8 h (T8). In both the T16 and T8 treatments, the number of sucklings was higher during the first reunited hours after separation (T16:16.00-19.00 and T8 08.00-09.00) than during the rest of the time ($P<0.05$). During the first reunited hours, the suckling frequency was almost as twice as high for T8 (3.9 ± 0.2 times) as in the T16 treatment (2.1 ± 0.2 times). This indicates that duration of separation affects the suckling behaviour, since the estimated milk consumption from both WSW and suckling behaviour observations demonstrated that the kids were more eager to suckle when they were separated from their mother for a longer time. Even if the kids had free access to hay and were fed restrictively with concentrate, they might have been hungrier after long separation (16 h) compared with short (8 h). Alternatively, the more frequent suckling during the first reunited hours might have been due to lack of nursing during the separation period and to both dams and kids expressing their relief on being reunited and therefore suckling more frequently.

In the T16 treatments, the number of suckling bouts declined at 19.00 and remained low until 05.00 (Fig. 16, left) and increased again during the last hour before separation ($P<0.05$), which is probably because nursing activity often has two major peaks, at dusk and dawn (Lent, 1974). Furthermore, during the last hour together regular feedings occurred and the goats had higher activity in general during these times (Högberg, 2008). The mean suckling duration per bout increased when the number of suckling bouts decreased, with the longest duration between 19.00 to 05.00. This pattern was not evident in the T8 treatment, where the duration was similar during all hours (Fig 16, right). The mean duration per bout was longer (P<0.05) for T16 (25 s, range 19-38 s) than for T8 (20 s, range 13-38 s).

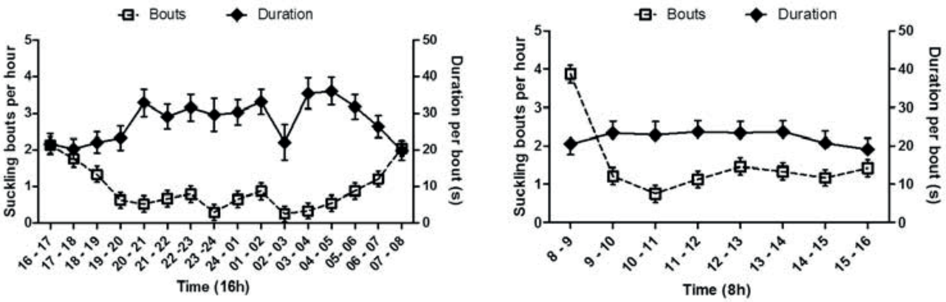

Figure 16. Number of suckling bouts and suckling duration per bout recorded between 16.00 h and 08.00h in the 16-h free suckling treatment (T16, left) and between 08.00h and 16.00h in the 8-h free suckling treatment (T8, right). The behaviour was recorded on day 4 once a week (2 days/treatment). Values are presented as least square means ± standard error of mean (SEM).

While the total time for free suckling was twice as long for T16 as for T8, there was no difference in the total number of suckling bouts between the treatments (Figure 17, left). The suckling frequency (bouts/h) was thus twice as high in the T8 treatment compared with T16, which shows that the kids compensated for their milk intake during the shorter period together (Figure 17, right). In addition, the duration per bout was shorter for T8 (20 ± 3 s), compared with T16 (25 ± 3 s). This indicates that the goats had more restless behaviour in the longer separation treatment, since they suckled more frequently and had a shorter duration per bout.

The number of suckling bouts in Swedish dairy goats seems to be equivalent to that in Tokara native goats, where the suckling frequency for 9 hours is reported to be 18.7 bouts during week 7 and 13.8 bouts during week 9 (Bungo *et al.*, 1998). In contrast to that study, there were only few suckling rejections in the goats in this thesis, whereas the Tokara breed rejected the kids from suckling 48 times during 9 hours. The difference in rejections could depend on housing, since our goats were kept in a free-range pen together with the rest of the herd, while the Tokara goats were reared in small individual pens. Since goats are gregarious, isolation from their social group can cause emotional stress (Kannan *et al*., 2002), which might lead to increased suckling attempts from the kids, which the dams probably reject, explaining the higher rejection rate in that study.

The suckling behaviour appears to vary greatly between individuals (Delgadillo, 1997; Cameron, 1998), even within the same treatment in the present thesis. For example, one kid suckled 29 times during 16 hours, whereas one other kid only suckled 8 times during the same period. Furthermore, the kids that suckled more frequently had a shorter duration per bout than kids that suckled less frequently. Dams and kids seemed to adapt to the treatments differently, with only five of 12 kids suckling more times during 16 h than 8 h, while the remainder (7/12 kids) suckled more often or as frequently during the shorter period together (T8) compared with T16. Another reason why kids suckled more during T8 (free suckling for 8 h during daytime) could be that they are generally more active during daytime than at night. During daytime, goats may spend more time on eating and social activity to retain social structure in the herd.

During recordings of suckling duration, it was found that the nursing behaviour can be divided into several parts. Therefore, time recording started when the kid had the teat in the mouth and started tail-flicking. In dairy cows, the first phase (pre-stimulation) consists of short sucking bouts associated with a relatively high frequency of butting; in the second phase (milk intake) sucking bouts are much longer and rhythmical and butting frequency is low; in the third phase (post-stimulation) sucking bouts are short and butting frequency is first high and then decreasing (Lidfors *et al*., 2010). The pattern might differ between goats and cows, since they have different udder morphology. Large amounts of fat-rich milk ejected earlier are already available in the large cistern of goats, whereas butting might be less frequent in kids unless the dam has low milk production or has more than one kid (less milk). In this thesis, kids preferred to suckle both teats (teat shifting) in about 90% of the total recordings. One goat suckled on the left teat only during all observations. This behaviour was probably due to the goats being machine milked twice daily for at least 12 weeks (enough milk yield for a single kid). We have earlier observed that single kids that suckled exclusively often preferred to suckle one teat only, especially when the dam was high yielding.

The dam disrupted the suckling session in 94 ± 2 % of the total observations during the day (T8) and in 92 ± 2 % at night (T16) of the total observations. The suckling sessions were only disrupted by the kids in 4 ± 2 % of cases during the day and 6 ± 2 % at night. Only 5.3 ± 2 % (T16) or 4 ± 2 % (T8) failed suckling attempts (dam rejected suckling) were observed.

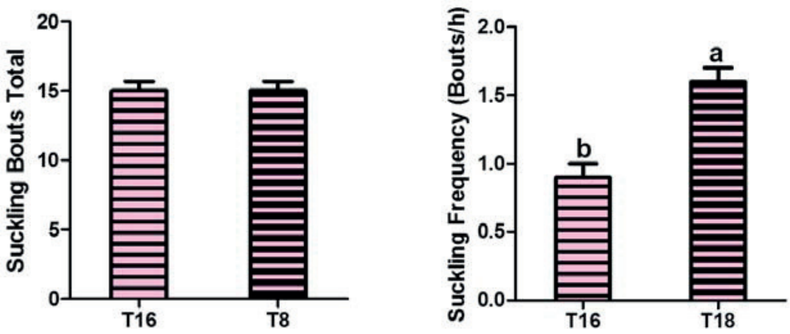

Figure 17. The total number of suckling bout (left) and suckling frequency (right) during 16h of free suckling (T16) and 8h of free suckling (T8).

As regards how partial suckling managements affect goats and kids, separation is usually considered a stressful event (Sevi, 2009), but the animals may experience the situation differently depending on several factors. Studies have shown that it may be less stressful to separate dams and kids in the beginning of the lactation. During the first 4 weeks after parturition, contact between dam and kid is infrequent, since goats are hiders and leave their offspring alone while *e.g*. going to pasture. During this period, dam and kid behaviours are not well synchronised (Lickliter, 1987), which is typical of species that use a hider strategy to protect newborns. This is in contrast to sheep, which instead use a follower strategy and show highly synchronised behaviour of dam and offspring from the beginning of lactation (Lickliter, 1984). Therefore, it could be more appropriate to use a partial suckling system for goats, since they might be less affected by the partial separation than other species that use a follower strategy. Moreover, kids tend to associate and form bonds with other kids of the same age, rather than with their mothers (Lickliter, 1987), and the formation of 'creches' might make the kids more stable if partial separation is practised.

The goats and kids used in this thesis were probably affected by the separation even though they were habituated to the procedure. Both dams and kids bleated, especially when the door between the dams' and kids' rooms was opened. Therefore, it might have been better to have the dams and kids in the same room, allowing them to receive visual and auditory stimuli (Orgeur *et al*., 1999). Moreover, in a study by Rassu *et al*. (2015), ewes and lambs showed a positive attitude to being temporarily apart and the task of separating the ewes from their young before going to pasture in the morning was performed easily. In addition, milkers observed calm behaviour and absence of bleating in ewes kept partially apart from their lambs during the evening milking. These positive elements of welfare evaluation were not verified in ewes milked only, who showed higher bleating activity and became more nervous during milking (Rassu *et al*., 2015).

Another tool to minimise stress is gentle handling and adapting young animals to solid food, thus counterbalancing lower milk availability with higher hay and concentrate intake (Sevi *et al*., 2009). When the kids in this thesis were separated, they were kept together with other kids and adults. In sheep, it is suggested to keep the weaned lamb in the presence of older and more experienced animals to help it to overcome the impact of separation from its mother (Sevi *et al*., 2009).

In conclusion, as suggested by Bungo (1998) and Miranda-de la Lama and Mattiello (2010), it appears that weaning of kids should not occur before 6-7 weeks of age. If goats are kept in dairy systems, partial suckling management might be preferable to total separation. Since goats are successful when colonising new environments, since they learn quickly and have excellent long-term memory (Briefer *et al*., 2014), which means that they probably remember that they will be reunited after a period of separation.

4.6 Mixed regimes, milk composition and curd yield (Paper IV)

Mixed management systems, based on both milking and suckling, have been shown to increase the milk yield available for processing compared with exclusive suckling regimes (Knight *et al*., 1993; McKusick *et al*., 2001). Recent observations in this thesis have shown that suckling can elevate milk fat concentration in goats. The last step was therefore to test whether the cheese processing properties of goat's milk can be optimised by appropriate management of suckled/milked goats (Paper IV).

It was found that both milking regime (Suckling before Milking = S; Milking before Suckling = M) and milk accumulation interval (Long/Short) influenced milk composition and cheese processing properties of goat's milk. Milk accumulation interval was the time for which dams and kids were separated. In T16 treatments, dams and kids were kept together for 16 hours and separated for 8 h (Short accumulation), while in the T8 treatments goats and kids were kept together for 8 hours and separated for 16 h (Long accumulation).

4.6.1 Milk production

Total milk production (milk off-take + WSW + suckled yield) was not affected by treatment. In fact, total milk production in Paper IV (Table 1, paper IV) was higher than the average milk yield in Swedish dairy goats (2.8 L/day, milk off-take only; Brandt, 2009), even though the milk offtake was lower. Several studies have shown that more frequent milking increases milk yield in goats (Henderson *et al*., 1985; Wilde *et al*., 1987; Koyuncu & Pala, 2008). Moreover, Wilde *et al* (1987) suggested that suckling plus frequent milking during early lactation can improve both mammary proliferation and differentiation of mammary cells of goats. Delgado-Pertínez *et al.* (2009a, 2009b) reported that dams kept with their kids for 18-20 h daily had higher milk production during the entire lactation (210 days) than dams that were milked only.

When measuring milk yield in dairy goats, the milk off-take from milkings is usually counted as the total milk production. In Paper IV, the total milk production was calculated as both milk off-take and suckled milk yield. The milk off-take differed between all treatments, with the highest yield in the Long accumulation treatment (T8-M) and the lowest yield in T16-S ($P<0.05$) (Table 4). During the free suckling periods, the estimated milk consumption was on average higher ($P<0.05$) in T16-S than in T8 (S and M), but not higher than in T16-M. There was no difference in total milk production (milk off-take + WSW + estimated milk consumption during free suckling) between the treatments. The individual variations were large within all treatments (Table 4). In the T8-S and T8-M treatments, the time for udder filling was longer and the milk off-take was greater after 16 hours than after 8 hours. The individual variation in milk off-take is great between animals. Multiparous goats yield more than primiparous (Peris *et al*., 1997; Salama *et al*., 2004; Carnicella *et al*., 2008) and disparities in cisternal capacity for storing milk seem to have major impacts on milk off-take (Marnet & Komara, 2008). It can be suggested that suckling is a stronger stimulus than milking and that complete removal of the alveolar milk, which is only achieved with complete milk ejection, is a prerequisite to maintaining a high level of milk synthesis and secretion throughout ongoing lactation (Bruckmaier & Wellnitz, 2008).

4.6.2 Fat concentration

The milk fat concentration differed between all treatments and was highest in T16-S and lowest in T8-M ($P<0.05$, Figure 18). Both milking regime and milk accumulation interval affected the milk fat concentration, which was higher when dams were suckled before milking (T16-S and T8-S) than when they were milked before suckling (T16-M and T8-M). Longer time together (T16-S and T16-M) also gave a higher fat concentration compared with T8-S and T8-M ($P<0.05$). The efficiency of transferring the fat-rich milk from the alveolar fraction into the cistern is related to plasma levels of OT, and for complete emptying of that milk, plasma OT concentration must reach a certain level (Shams *et al*. 1984; Bruckmaier *et al*., 1994a). Keeping dams and kids together could therefore improve milk removal from the alveoli by more regular OT release, since more frequent milk ejection increases the proportion of fat-rich alveolar milk, ultimately increasing the total milk fat concentration (Knight & Dewhurst, 1994; Negrao & Marnet, 2003).

The significant reduction in milk fat in the T8-M treatment confirmed that cisternal milk was mostly obtained during those milkings (McKusick *et al*., 2002a; Paper III). Comparable findings have been observed in dairy ewes kept in mixed management systems (McKusick *et al*., 2001, 2002a; Jaeggi *et al*., 2008; Tzamaloukas *et al*., 2015), which has been explained by impaired milk ejection during milking caused by partial contact between dam and lamb (McKusick *et al*., 2002a). The management design for T8-M and the studies by McKusick *et al*. (2002a) and Jaeggi *et al*. (2008) was similar. Dams and offspring were separated for 14 to 16 hours at night and milked once daily without udder stimulation by the offspring before milking, which explains the low fat concentration. Similarly low fat concentrations were found in Paper II, where the goats were milked before suckling (after a period of separation). These kinds of milking regimes might lead to deficient OT release, where only cisternal milk is available during milking (Cetin *et al*., 2010), as demonstrated in Paper II.

In contrast, Peris *et al*. (1997) showed that the fat concentration was similar between dams reared in mixed regimes compared with goats milked only. Delgado-Pertinez *et al*. (2009a, 2009 b) showed only small differences in fat concentration between milked and suckled goats. In both those studies, sucking was performed before milking and the kids were separated for some hours before each milking, as in this thesis. The results clearly show that management system has a major impact upon milk fat concentration. Based on the present work, suckling before milking and shorter separation between dam and kid can be recommended when rearing dams and offspring in mixed management systems.

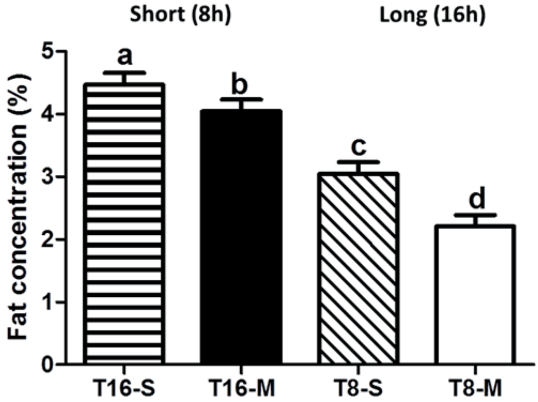

Figure 18. Milk fat concentration in milk from goats assigned to 4 different treatments (cross-over design); two milk accumulation intervals (Short, Long) and two milking regimes (Suckled Before Milking = S, Milked Before Suckling = M). In the short accumulation treatment, dams and kids were separated for 8 h in daytime and together for 16 h at night (T16). In the Long accumulation treatment, dams and kids were separated for 16 h at night and together for 8 h in daytime (T8). Values are presented as least squares mean (LSM) ± standard error of mean (SEM). Different superscript letters indicate significant differences (P < 0.05).

4.6.3 Suckling and somatic cell count (SCC)

Somatic cell count was higher in both T8 treatments than in the T16 treatments (P<0.001) and T8-S had even higher SCC than T8-M (P<0.05). The variation in SCC between individuals was large (Figure 19). It has been debated whether suckling has an improving effect on udder health. Rigby *et al.* (1976) suggested that suckled cows have a lower incidence of mastitis, due to better udder emptying and bacterial inhibitors in the saliva. Nevertheless, some people believe that suckling can increase the risk of infection spread because of cross-suckling. However, this is probably very uncommon in goats, since they are normally attached to their own offspring only (Hernandez *et al.*, 2002). In general, goats normally have much higher SCC than sheep and cows. The reported values vary between breeds, regions, parity and stage of lactation (Paape *et al.*, 2007). The rise in SCC during T8 (S and M) treatments in Paper IV may be explained by the abrupt change from 8 to 16 hours of free suckling, since when the udder undergoes less frequent evacuation, the SCC tends to rise (McKusick *et al.*, 2001). This increase in SCC was more apparent in dams with generally higher SSC values than in dams with lower SCC, where only small or no changes were observed. One individual differed greatly from others, with SCC >1000 x10^3cells/mL (thereby the high range), but no bacterial infection or difference in milk composition or milk pH was found in that goat. The individual SCC for the total period (all treatments) differed between animals.

Four dams had SCC <100 x10^3cells/mL, four dams had SCC between 100 and 300 x10^3cells/mL, three dams between 700 and 900 cells/mL and one dam had >1000 x10^3cells/mL.

However, the SCC in T16 (S and M) was lower than in other goat breeds (Paape *et al*., 2007), and surprisingly much lower than in the closely related Norwegian dairy breed, which has >1000 x 10^3 cells/mL in normal milk (Skeie, 2014). The present SCC values might indicate that suckling has beneficial effects upon udder health (Fröberg *et al*., 2008) or may confirm that Swedish goats have good udder health status in general (Persson & Olofsson, 2011). Moreover, Rassu *et al*. (2015) observed that ewes suckled by their lambs had lower SCC levels than other ewes within the same breed. Others have also reported that ewes reared in combined suckling-milking management have lower SCC levels than dams milked only (McKusick *et al*., 2001, 2002a; Tzamaloukas *et al*., 2015). In goats, no difference in SCC has been observed between naturally sucked dams and dams milked only (Delgadado-Pertinez *et al*., 2009a, 2009b). Even though cisternal milk was mainly obtained in the T16 (S and M) treatments in Paper IV, the SCC was higher in those treatments. In contrast, McKusick *et al*. (2001, 2002a) and Sarikaya *et al*. (2005) demonstrated that the alveolar and residual fractions contain higher SCC than cisternal milk in sheep and cows.

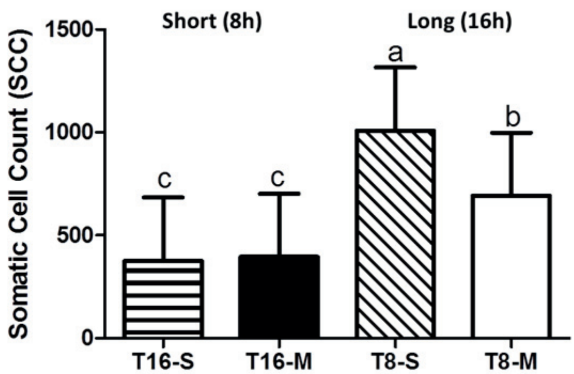

Figure 19. Somatic cell count in milk from Swedish dairy goats assigned to four treatments; two milk accumulation intervals (Short, Long) and two milking regimes (Suckled Before Milking = S, Milked Before Suckling = M). In the short accumulation treatment, dams and kids were separated for 8 h in daytime and together for 16 h at night (T16). In the Long accumulation treatment, dams and kids were separated for 16 h at night and together for 8 h in daytime (T8). Values are presented as least squares mean (LSM) ± standard error of mean (SEM). Different superscript letters indicate significant differences (P < 0.05).

4.6.4 Casein concentration

The casein concentration was significantly higher ($P<0.001$) when dams and kids were together for a longer time (T16) than when they were together for a shorter period (T8), which also resulted in higher casein number (casein/total protein) in T16 compared with T8 ($P<0.05$) (Figure 20). These results indicate that accumulation interval, such as longer time spent together, had a higher impact on the casein concentration than milking management (S or M). When dams and kids are reared jointly for longer times (16 h), both udder stimulation and OT release might be more frequent than when they are together for 8 h. Lollivier *et al.* (2006) demonstrated *in vitro* that OT stimulates intracellular transport off newly synthesised caseins (α_{S1}-CN) and suggested that the emptying of the mammary epithelial cells might avoid negative feedback of accumulation of the milk constituents and may stimulate synthesis of new milk protein. This might explain the higher casein concentration in the T16 treatments compared with T8. The casein number followed the same pattern as the casein concentration. Jaeggi *et al*. (2003) showed that the casein fraction was lower in milk with high SCC, which could also have been the case in the present study.

On the other hand, the higher casein concentration in the T16-S and T16-M treatments could be a result of more concentrated milk (Salama *et al*., 2003), since these treatments had a lower milk off-take at milking. However, milk protein and casein are less affected by milking management (Capote *et al*., 2008) since the molecules are small and pass freely between the alveolar and cisternal fractions (Wilde *et al*., 1996; Salama *et al*., 2005; Capote *et al*., 2008; Koyuncu & Pala, 2008). It is well accepted that the casein concentration in milk is highly correlated with the genetic profile (Park *et al*., 2007), and higher casein is associated with a higher proportion of α_{S1}-CN in milk, a higher content of total solids and improved cheese processing properties in general (Ambrosoli *et al*., 1988). The higher casein concentration observed in Paper IV was probably not related to the genetic casein profile, since the experiment was a cross-over design where all goats received the same treatment. It has recently been reported that the casein concentration and proportion (α_{S1}-CN) can also be influenced by dietary treatment (Inglingstad *et al*., 2014), but the results in this thesis suggest that more regular udder stimulation and milk removal might stimulate protein synthesis, as suggested previously by Lollivier *et al.* (2006).

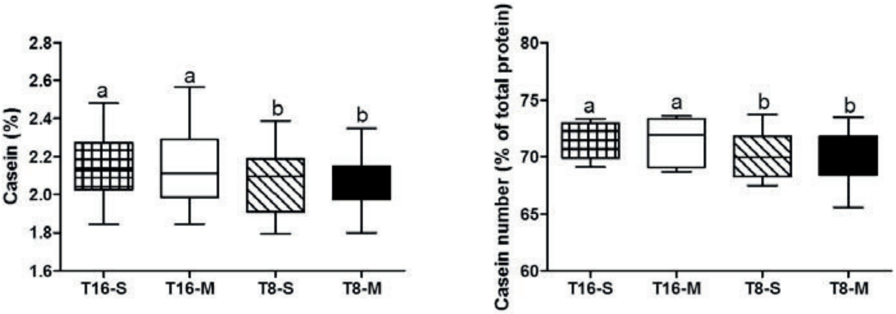

Figure 20. Casein concentration (left) and casein number (casein/protein, right) in milk from Swedish dairy goats assigned to four treatments; two milk accumulation intervals (Short, Long) and two milking regimes (Suckled Before Milking = S, Milked Before Suckling = M). In the short accumulation treatment, dams and kids were separated for 8 h in daytime and together for 16 h at night (T16). In the Long accumulation treatment, dams and kids were separated for 16 h at night and together for 8 h in daytime (T8). Values are presented as least squares mean (LSM) ± standard error of mean (SEM). Different superscript letters indicate significant differences ($P<0.05$).

4.6.5 Individual Curd Yield

The individual curd yield differed between milking regime, milk accumulation interval and goats. The curd yield was highest in milk samples with a high fat and casein concentration in general (Figure 21, left).

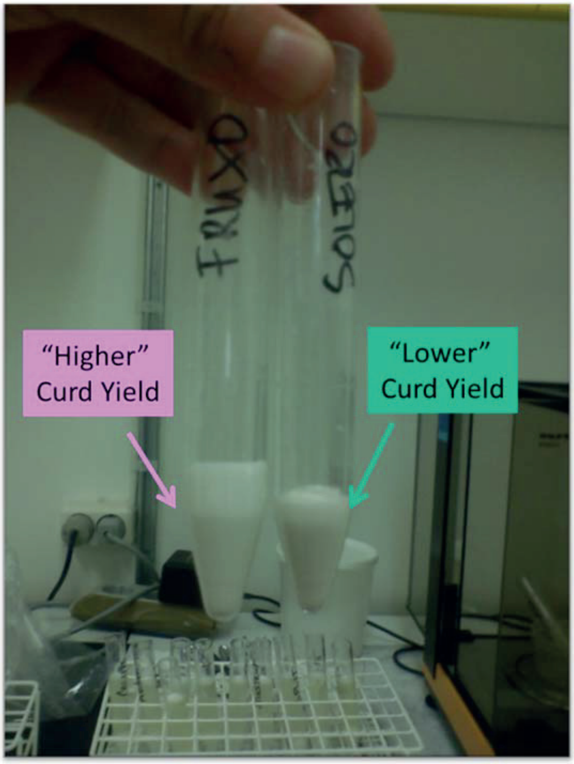

Figure 21. The individual curd yield was higher in the left tube than in the right. Exactly 10 mL of milk were pipetted into each tube before rennet was added.

The results in Paper IV showed that suckling before milking increased both milk fat concentration and curd yield. This clearly demonstrates that suckling improves the neuroendocrine milk ejection reflex (Marnet & Negrao, 2000; Paper II) by releasing the alveolar milk rich in fat into the cistern (Linzell & Peaker, 1971). The individual curd yield (%) was higher in T16 than in the T8 treatments ($P<0{\cdot}001$; Figure 22, left). Comparing suckling (S) and milking (M) management, the curd yield was higher in T8-S than in T8-M and tended to be higher in T16-S than in T16-M ($P=0.07$). There was no difference in the total individual curd yield (yield % x milk off-take) between the treatments (Figure 22, right).

The significantly higher fat and casein concentration in the T16 treatments resulted in a higher curd yield compared with the T8 treatments (Figure 22). High fat content increasing the curd yield has also been reported by others (Soryal *et al*., 2005; Jaeggi *et al*., 2008). The individual curd yield was only 7.5% in T8-M, compared with about 14% in milk from T16-S and T16-M treatments. The cheese yield from Swedish dairy goats is in general 10-12% (10-12 L milk to produce 1 kg of hard cheese; M Leipage, pers. comm. 2010), which is similar to the curd yield in the T8-S treatment. It should be mentioned that milk samples with a fat concentration higher than 5% were excluded from the analysis of individual curd yield, due to missing values. The individual curd yield was therefore underestimated and higher values would have been obtained if all samples from the T16 treatments had been included. When comparing the total individual curd yield in grams (% x milk off-take), there was no difference between treatments. This confirms that the milk composition has a greater impact than the yield itself when milk is intended for use for cheese production.

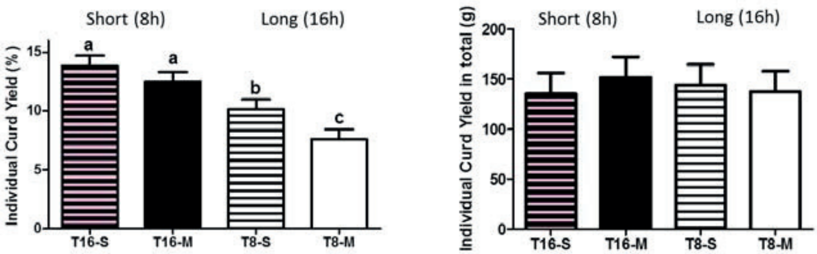

Figure 22. Individual curd yield per day and goat as a percentage (left) and total individual curd yield in grams (milk off-take x %, right). Values are presented as least squares mean (LSM) ± standard error of mean (SEM). Different superscript letters indicate difference ($P<0.05$).

4.7 Further observations

During the course of this work, further observations were made that were not included in the papers, but could be of interest and are therefore included in the following sub-sections as previously unpublished results.

4.7.1 Two different methods of measuring casein concentration

Before my earlier studies (Högberg, 2011a, 2011b) the casein concentration in Swedish Landrace goats was unknown. In the present thesis, the methodology for analysing the total casein concentration was further advanced to fit both laboratory and on-farm analyses.

It can be problematic to analyse the casein concentration on-farm by the centrifugation method, so an existing filtration method was tested to see whether it gave the same results as the centrifugation method. The two different methods were tested for reliability, using dual sub-samples (n=35 x 2) from the same milk sample (Figure 23). The results from the different tests were almost identical, with only two samples showing different results. The centrifugation and filtration methods were thus highly correlated (R^2 = 0.8, P<0.0001), and both can be used when analysing the casein concentration in the laboratory or on farms.

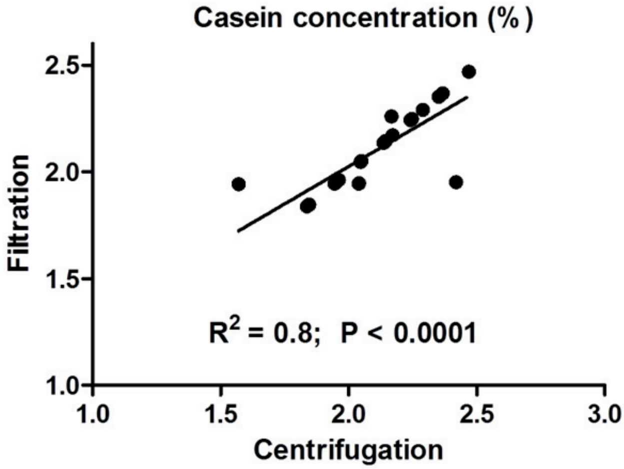

Figure 23. Comparison between two different methods (filtration and centrifugation) when analysing the casein concentration in milk.

4.7.2 Casein concentrations during different stages of lactation

During farm visits, some cheese producers mentioned difficulties during cheese processing when the milk came from early lactation. The first suggestion was that it might depend on the proportion of casein and whey protein in the milk. The casein concentration was therefore analysed at different stages of lactation with the aim of determining whether the casein number differed between early and late lactation.

The results showed that the casein concentration was higher in the beginning of lactation (day 14) compared with later stages (days 27, 31, 62 and 65) (Figure 24, left). The casein number was lowest in the beginning of lactation and increased over time (Figure 24, right). The low casein number in the beginning of lactation might be explained from a physiological perspective by the casein and whey levels changing as lactation progresses to meet the nutritional needs of the offspring. The whey fraction consists of β-lactoglobulin, α-lactalbumin, serum-albumins and immunoglobulins (Walstra, 1999), of which the last play a very important role in immunological defence for the offspring.

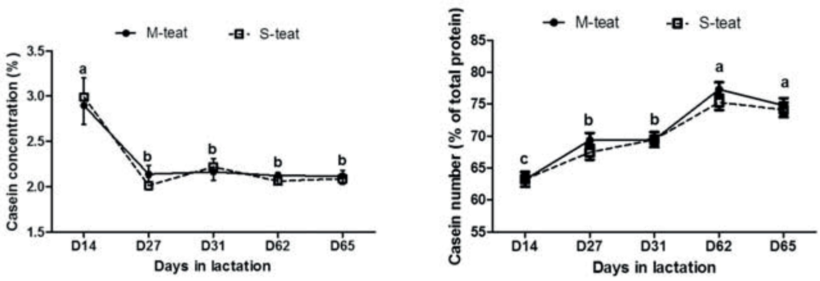

Figure 24. Total casein concentration (left) and casein number (casein/protein in percent, right) during different days of lactation (D14- D65).

4.7.3 Adaption to milking

The main finding in this regard was that early adaptation to milking might have beneficial effects during machine milking of mixed reared goats. When the goats were adapted to milking in an early stage of lactation (on lactation day 5), both cisternal and alveolar milk yield was higher than when they were adapted to milking at later stages of lactation (about 4 weeks, Table 4). The milk fat concentration in the cisternal milk fraction was twice as high when dams were adapted to milking early as when adaptation to milking occurred in later stages of lactation. This indicates that both cisternal and alveolar milk was obtained when milking dams adapted early. When adaptation occurred later, it is likely that only cisternal milk was obtained during milking, whereas milk ejection only occurred when the dams were adapted early. It has further been demonstrated that ewes adapted to machine milking had higher OT levels, higher milk yield and milk fat than ewes not adapted to milking (Negrao & Marnet, 2003).

Early adaptation to milking is not always the case for dairy animals kept together with their offspring in the beginning of lactation. For example, dairy ewes are often suckled freely by their lambs without being milked in the beginning of lactation (Marnet & Komara, 2008). This type of management has also been observed in Swedish dairy goats and sheep, which might affect the milk yield negatively, in particular for animals that give single births. High yielding animals often produce more milk than the offspring can consume (Marnet & Komara, 2008), which has also been observed in the goat flock at SLU. When dams and kids were kept together exclusively, kids mainly preferred to suckle one teat only, while the other udder half dried off. When the goats were kept together with one kid each, kids preferred to suckle both teats as long as the dams were milked once or twice daily.

Only 43-44% of the total milk volume was obtained from the cisternal fraction, which can be explained by the short accumulation time (3 h). If the accumulation interval had been longer, a higher proportion of milk would almost certainly have been obtained from the cisternal fraction. It is not clear if Swedish dairy goats have large or medium-sized cisterns, since there are no scientific data on cisternal size in Swedish dairy goats. From this thesis, it can be concluded that dams kept in mixed management regimes should be adapted to milking in an early stage of lactation. Early adaptation to machine milking is also recommended, to prevent failure in milk ejection.

Table 4. *Milk yield and fat content in milk from dams adapted to milking late (4 weeks) or at an early stage of lactation (5 days). One teat was milked only, after 3 h of accumulation.*

		Mixed managements		
		Late adaption to milking	Early adaption to milking	Late adaption to milking
	Animals	n = 8	n = 8	n = 8
	Lact. Number (mean)	1.4	2.3	2.8
Milk yield (g)	Cisternal	88 ± 6	270 ± 24	102 ± 13
	Alveolar	118 ± 6	286 ± 24	129 ± 13
	Total	206	556	231
	CM proportion (%)	43	49	44
	AM proportion (%)	57	51	56
Milk fat (%)	Cisternal	1.8 ± 0.17	3.9 ± 0.3	2.6 ± 0.3
	Alveolar	3.6 ± 0.17	5.9 ± 0.3	4.2 ± 0.3

Behaviour recording during the milking procedures revealed that with late adaptation to milking, none of the animals started to ruminate, whereas nearly all goats that were adapted to milking early started to ruminate in connection with cisternal milking. During natural suckling, goats normally ruminate during the suckling episode, which is an indication that the alveolar milk is ejected (Andersson *et al*., 1958). With this in mind, the types of milking management/routines that are best fitting for the dairy goat can be considered. Goats are mainly milked in connection with feeding, and the rate of milking is often very fast. It is common for research to focus on different techniques to improve the milk flow and achieve faster milking, which is believed to be more efficient and economical, since it saves more time for the farmer. It is possible that faster milking leads to deficient milk ejection in some goats and that a different milking routine, *e.g.* longer time for milking, would improve both milk yield and composition. It has been shown that OT is released in response to feeding in dairy cows (Svennersten *et al*., 1990), which thereby increases milk yield, but it is not clear if OT increases in goats during feeding. Goats can be very eager during feeding, which might inhibit OT release from the pituitary. Therefore, it might be advantageous to extend the milking procedure, for example by starting pre-stimulation after feeding.

Another important factor that can influence milk production is reducing the emotional and physical stress of dairy animals (Sevi *et al*., 2009). Gentle handling and positive physical attention to the animals can increase the probability of a healthy human-animal bond, which in turn can have positive effects on animal health and milk production (Miranda-de la Lama & Mattiello, 2010).

5 General conclusions

From the results of this thesis it can be concluded that;

- A high frequency of Swedish Landrace goats produces low amounts of α_{S1}-CN, which can affect the cheese making properties negatively.
- Plasma levels of both oxytocin and vasopressin increased during suckling, but not during milking. In a mixed milking-suckling system, presence of the kids may be necessary to stimulate release of peptides and contraction of the myoepithelial cells.
- Milk yield and fat concentration increased after both oxytocin and vasopressin administration, which demonstrates that vasopressin can cause a milk ejection reflex similar to the oxytocin response.
- A milking regime with suckling before milking increased milk fat concentration and curd yield compared with milking before suckling.
- Milk fat, casein concentration and individual curd yield (%) was higher when dams and kids were together for a longer time (16h) compared to when they were together for a shorter time (8h).
- Animal welfare is most likely higher when dams and kids spend more time together.

6 Populärvetenskaplig sammanfattning

Intresset för getost och lokalproducerade livsmedel har resulterat i att antalet getföretag och svenska lantrasgetter har ökat kraftigt under senare år. Denna utveckling är betydelsefull för den svenska landsbygden och getnäringen och fyller en viktig funktion för utveckling och nyskapande av arbetstillfällen. Den svenska getosten produceras vanligtvis i egna gårdsmejerier där mjölkens sammansättning, framförallt fett, protein och kasein har en avgörande betydelse för ostutbyte och kvalité. Flertalet getraser, inklusive den svenska lantrasgeten, har en relativt låg andel fett, protein och kasein i mjölken, vilket kan leda till försämrade ystningsegenskaper och ge ett lägre ostutbyte som följd. I dagsläget finns inga studier gjorda på den svenska lantrasgetens genetiska variation i mjölksammansättning, eller på hur olika mjölkningsrutiner påverkar mjölksammansättning och ostutbyte. I denna avhandling har därför faktorer som är betydelsefulla för att få en lönsam, hållbar getostproduktion med bra djurvälfärd undersökts.

6.1 Bakgrund

Den svenska getmjölkens protein består av ca 72 % kasein (ostämne) och 28 % vassleprotein (restprodukt vid osttillverkning). Kaseinets sammansättning delas i sin tur in i α_{S1}-kasein, α_{S2}-kasein, β-kasein och Ϗ-kasein. Det har visat sig att andelen kasein och kaseinsammansättning har stor betydelse för mjölkens ystningsegenskaper. Mjölk som innehåller högre kaseinhalt och har högre andel α_{S1}- och Ϗ-kasein påverkar ystningsegenskaperna positivt. Eftersom α_{S1}-kaseinerna har en betydande roll vid osttillverkning har forskning gjorts på kaseininnehåll i getmjölk. Individuella getter kan klassificeras genetiskt genom syntes av ”stark, medel, svag eller noll” syntes av α_{S1}-kasein. De norska lantrasgetterna tillhör en population där vissa individer genom en mutation tappat förmågan att producera α-$_{S1}$-kasein. Andelen ”noll”-variant uppmättes i en studie till över 70 %, vilket påtagligt minskar mjölkens kaseinhalt och ostutbyte. Eftersom svenska lantrasgetter är nära besläktade med den norska lantrasen är det sannolikt att även svenska getter bär på anlaget (genen) för noll-syntes av α-$_{S1}$-kasein. Syftet med det första arbetet var därför att kartlägga andelen olika kaseinvarianter hos svenska lantrasgetter.

Mjölkens fetthalt är en annan viktig faktor som påverkar ostutbytet. Till skillnad från proteinet påverkas mjölkens fetthalt i större utsträckning av exempelvis utfodring, mjölkningsrutiner, juvermorfologi, och digivning. De tre sistnämnda har betydelse vid själva mjölkningen där frisättningen av hormonet oxytocin (mjölknedsläppningsreflexen) har stor betydelse för mjölkens mängd och fetthalt. I de två efterföljande arbetena studerades hormonerna oxytocin och vasopressins roll för digivning och mjölknedsläpp hos getter. Idisslare lagrar den nybildade mjölken i juvrets alveoler och cisterner, varav cisternmjölken kan mjölkas ur utan mjölknedsläpp. Den fettrika mjölken som lagras i alveolerna blir tillgänglig först efter oxytocinets hjälp. Getter har till skillnad från kor stora cisterner där upp till 80 % av mjölken lagras, vilket innebär att getter kan mjölkas utan mjölknedsläpp. Detta kan till viss del förklara varför getter har lägre fetthalt i mjölk. Det har i tidigare studier visats att digivning kan ge en högre oxytocinfrisättning än mjölkning, vilket i sin tur leder till en bättre juvertömning och en högre fetthalt som följd. Det fanns därför anledning att vidare undersöka om digivning kan öka mjölkens fetthalt hos Svenska lantrasgetter.

Det pågår en allmän debatt om huruvida djur som föds upp inom mjölkproduktionen har möjlighet att utföra naturliga beteenden, då avkomman vanligtvis separeras direkt eller några dagar efter födseln. Från producenternas perspektiv anses det i många fall bli för stora ekonomiska förluster om mor och avkomma får gå tillsammans. Det har även visats att mjölkens fetthalt blir lägre av att ha tacka och lamm tillsammans, eftersom det anses att det lakterande djuret endast får mjölknedsläpp när avkomman diar, inte vid mjölkning. Inom den svenska getnäringen varierar dock tidpunkten för separation från några dagar till månader. Syftet med sista arbetet var därför att undersöka hur mjölknedsläppet påverkas av digivning samt att närmare utreda hur mjölksammansättning och ostutbyte påverkas av digivning när getter och killingar går tillsammans i olika system.

6.2 Sammanfattning av studier och resultat

Det första arbetet undersökte svenska getters kaseinprofil och mjölkprover togs från olika gårdar i Sverige. Det visade sig att av 283 getter hade 65 % låg eller ingen syntes av α_{s1}-kasein, vilket innebär att flertalet getter hade mjölk med sämre ystningsegenskaper. I nuläget är det därför viktigt med fortsatt kartläggning av svenska getters kaseinvarianter i mjölk, för att kunna bedriva ett avelsarbete som är lämpligt för en hållbar svensk getostproduktion i framtiden. Å andra sidan är populationen av svenska lantrasgetter klassificerad som utrotningshotad, därför bör försiktighet vidtas om man endast selekterar genetiskt för individer med bra kaseinsammansättning. Det är därför av stor vikt att undersöka andra faktorer som kan påverka mjölkens ostutbyte, som till exempel mjölkens fetthalt. Resterande arbeten i denna avhandling undersökte därför hur fetthalten påverkas ur ett fysiologiskt perspektiv, i detta fall genom mjölknedsläpp och digivning.

Det andra arbetet i avhandlingen visade att under digivning ökade hormonet oxytocin samtidigt som det närbesläktade hormonet vasopressin, men detta skedde inte under mjölkning. Detta tyder på att getter som går tillsammans med sina killingar endast släpper ner mjölken till sina killingar och inte till mjölkaren. Att hormonet vasopressin ökade under digivning var spännande i sig, eftersom vasopressin mest är känt för sin antidiuretiska effekt som hjälper kroppen att spara vätska vid brist på vatten och uttorkning. Detta är en viktig upptäckt eftersom många getter runtom i världen lever i karga förhållanden med stundvis brist på vatten. De båda hormonerna är närbesläktade och kan binda till varandras receptorer, vilket i praktiken innebär att båda hormonerna skulle kunna medverka till mjölknedsläpp, därför gjordes det tredje arbetet. Resultaten i det arbetet visade att både mjölkmängd och fetthalt ökade efter både vasopressin- och oxytocinbehandling, vilket tyder på att vasopressin kan orsaka mjölknedsläpp på ett liknade sätt som oxytocin.

I det sista arbetet undersöktes hur mjölkens fetthalt påverkades av digivning i ett system där get och killing går tillsammans. Det visade sig att mjölkens fetthalt liksom oxytocin ökade under digivning, men ingen ökning sågs under mjölkning. Detta var ytterligare ett bevis på att getterna endast släppte ner mjölken till sin killing och att digivning gav en bättre juvertömning jämfört med mjölkning. Eftersom fetthalten var låg vid mjölkning och endast cisternmjölken var tillgänglig var då frågan hur den positiva juverstimuleringen från killingen skulle kunna användas i praktiken om getter och killingar går tillsammans? Därför gjordes ytterligare studier för att närmare undersöka detta.

När djur inom mjölkproduktionen hålls tillsammans med sin avkomma är det möjligt att separera moderdjur och avkomma några timmar per dag för att spara mjölk till mejeriet, dvs. använda en restriktiv digivning. I sådana system är det också vanligt att själva mjölkningen sker i direkt anslutning till separationen och att djuren mjölkas innan digivning. I sista arbetet hölls därför getter och killingar tillsammans i fyra olika system med restriktiv digivning. I två system hölls getter och killingar tillsammans under 16 timmar nattetid och separerades under 8 timmar dagtid. I de andra två systemen hölls getter och killingar istället tillsammans under 8 timmar dagtid och var separerade under 16 timmar nattetid. För att testa mjölkningsprocedurens inverkan på mjölknedsläppet testades ytterligare två behandlingar inom de två systemen. I ena behandlingen diades getterna innan mjölkning och i den andra behandlingen mjölkades istället getterna innan digivningen (det vanligaste alternativet). Mjölkens fetthalt, protein och kaseinhalt analyserades för att undersöka om mjölkens ostutbyte påverkades av de olika systemen. En metod för att med mäta individuellt ostutbyte utvecklades. Studien visade att både fetthalt och ostutbyte blev högre om getterna diades innan mjölkning. Det visades även att kortare separationstid hade positiv inverkan på både fett, kasein och ostutbyte. Om man jämför dessa resultat med den svenska getmjölkens sammansättning generellt (ca 3,4 % fetthalt), så tycks digivning ge en högre fetthalt (4,5 %) och därmed ett högre ostutbyte.

Avslutningsvis tyder resultaten från denna avhandling på att mjölkproduktion och mjölksammansättning påverkas positivt av att getter och killingar hålls tillsammans. En för get och killing mer naturlig djurhållning är dessutom att rekommendera ur ett djurvälfärdsperspektiv.

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