Effects of Social Separation on Cortisol, Milk Yield and Composition, Udder Health and Behaviour in Dairy Cattle

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The present thesis is a partial fulfilment of the requirements for a Master of Science Degree in Veterinary Medicine for International Students at the Swedish University of Agricultural Sciences (SLU), in the field of Animal Hygiene and Ethology

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To my father Carlos Hernández, who gave me strong roots to grow tall

To my mother Maria de los Angeles Verduzco, who gave me wings to fly

To my sisters Melisa and Angelica, who gave me unconditional support

To my nephew Erick, who brought light to my life

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Abstract

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This thesis is based upon two studies carried out at a commercial organic dairy farm, in the southwest of Sweden.

The aim of the first study was to evaluate the effects of suckling and subsequent weaning after a nursing period of 9 weeks in foster cows with previous high somatic cell count (SCC) on their saleable milk yield (total milk minus discarded milk due to a high California Mastitis Test score), milk composition, udder health and behaviour during milking. Seven foster cows (calves suckling) and seven control cows (with no calves suckling) were used in the experiment. Four alien calves had suckled each foster cow for 9 weeks and the calves were free to suckle at any time. Behavioural observations were made during afternoon milking and milk samples were taken at 0, 1, 2, 3, 7, 10, 14, 17, 21 and 24 days after weaning. The saleable milk yield of the foster cows was significantly lower than the control cows during the first ten days after weaning. One day after weaning, the foster cows moved significantly more during milking and vocalizations were recorded (as yes or no) in five foster cows while none of the control cows vocalized. Four of the seven foster cows had mastitis towards the end of the study. The fat content in strip milk was significantly lower in the foster cows than in the control cows on the day of weaning, indicating a disturbed milk ejection. However, the day after weaning no such difference was observed. The foster cows had a significantly lower fat content in composite milk on the day of weaning. This fat content increased rapidly, and 10 days after weaning the foster cows had significantly higher fat content in composite milk than the control cows.

In conclusion, the suckling by four calves on foster cows with high SCC or mastitis, resulted in a higher fat content in composite milk after weaning. However, after 9 weeks nursing their udder-health condition did not seem to improve and the saleable milk yield was lower than in the control group. Furthermore, the weaning in addition to the beginning of milking seem to be a stressful situation as indicated by cows vocalizing more and moving more during milking.

The aim of the second study was to investigate the relationship between plasma and salivary cortisol response to stress in dairy cattle. To activate the cortisol stress response, six cows were separated from their calves at 4 d after calving, and six calves were separated from a group of four peers at 8 wk of age. In addition, all the animals were moved to an unfamiliar surrounding after the social separation. Jugular catheters were placed on the animals 1 d before sampling, blood and saliva samples were taken simultaneously before and after separation. Samples were taken at a 10 min interval during the first 2 h and further samples were taken every 15 min. In response to the stressors, there was an increase in plasma cortisol reaching peak mean values 25 min after the first sample. However, peak values in saliva cortisol in cows and calves were observed 35 min after the first sample. Plasma cortisol correlated well with saliva cortisol ($r = 0.626$). However, plasma cortisol explained more of the variation in the cortisol concentrations from saliva samples taken 10 to 15 min after (AIC = 727.0; $P < 0.001$) than in the saliva samples taken at the same moment as the plasma samples (AIC = 810.7; $P < 0.001$) and no significant effects of breed or group (cow or calf) were found in the models. The correlation coefficient for plasma and saliva-lag was $r = 0.744$. These results suggest a time lag between plasma cortisol values and associated changes in saliva cortisol values in dairy cattle.

In conclusion, salivary cortisol assessment seems to be a valid method to detect responses of the HPA axis following acute stress in cows and calves. This study suggests a time lag between plasma and saliva cortisol values and this should be considered when using saliva samples as the only measure of the stress response in cattle.

Keywords: Dairy Cow, Calf, Suckling, Milking, Unfamiliar Surroundings, Weaning, Cortisol, Plasma, Saliva, Social Separation

General background

Cow-calf bond

Maternal behaviour is a highly integrated mechanism essential for the survival of the young and the growth of the offspring. At least two essential components can be differentiated in the maternal behaviour of cattle; the care the females give to the young and their selectivity which limits that care to specific young (Le Neindre *et al*., 1998). Maternal behaviour is one of the major expressions of the interactions of the individual with its social environment (Le Neindre *et al*., 1998).

The quick forming of stable bonds between mother and young is essential. Young are often helpless, vulnerable to starvation, predation and cold, so their survival and fitness rely largely on the ability of the parents to rightly asses their needs and adjust the behaviour accordingly (Jensen, 2001).

The same kind of social interaction may occur when alien calves are introduced to the foster cows shortly after parturition and these cows might accept and develop a maternal bond towards the calves (Hudson, 1977). Introduction of the alien calves later in lactation does not seem to affect the acceptance of the alien calves by the foster cow. Loberg & Lidfors (2001) have recently shown that cows accept equally well alien calves regardless of the time after separation from their own calf (directly, 4, 26 and 178 days after). However, some problems may arise and the farmer might have to tie some cows to allow the calves to suckle for a few meals before they accept being suckled (Hudson, 1977; Lidfors, 2000).

Lactation

Lactation is central to mammalian reproduction and critical for survival of the neonate. It involves morphological, physiological, biochemical, ecological and behavioural adaptations (Blackburn, Hayssen & Murphy, 1989). Lactation consists of two phases: milk secretion or synthesis, which is controlled in part by a hormonal complex originating in the anterior pituitary, and milk removal or ejection, which is controlled primarily by the release of the hormone oxytocin from the posterior pituitary (Gorewit *et al*., 1983).

The growth of the mammary ducts is stimulated by oestrogen, and the lobule-alveolar development is stimulated by a combination of oestrogen and progesterone. These steroid hormones require prolactin and (or) growth hormone to stimulate mammary gland development and to initiate lactation (for a review see Tucker, 2000). In the later part of gestation the alveolar cells differentiate, and close to parturition secretion of milk is initiated (Tucker, 1981).

Milk synthesis and milk composition

The cow's udder is composed of two pairs of mammary glands, each drained by a teat. The four quarters are structurally separate and function independently (Gruet *et al*., 2001). The mature mammary gland consists of a teat, associated ducts that provide for passage of milk to the outside, and alveoli composed of epithelial secretory cells and supportive tissues. The epithelial cells are arranged to form the internal lining of the spherical alveoli, and cells synthesize and secrete all milk. Secretions are stored within the internal space of the hollow alveoli and larger ducts between suckling episodes (Nickerson, 1992). The synthesis and release of milk constituents is continuous, until temporarily suspended by the distending pressure (Gruet *et al*., 2001).

Glucose, acetate, β-hydroxybuturate, fatty acids and amino acids originating from blood are the major precursors of milk (Akers, 2002). These precursors are absorbed from blood capillaries (adjacent to alveoli) by the alveolar epithelial cells and some are converted into milk protein, lactose and fat (Nickerson, 1992).
Milk fat and milk protein are both synthesized in the endoplasmatic reticulum, while lactose is synthesised in the Golgi system (Davies, Holt & Christie, 1983). The dominant carbohydrate in the milk of most mammals is the disaccharide lactose and it occurs only in mammalian milk and in mammary glands (Michael & Tadasu, 2002).

The milk from Holstein cows (the source of the majority of milk for human consumption in Western societies) has about 3.2% protein, 3.4% fat and 4.6% of lactose (Akers, 2002). However, there are variations in the milk composition due to breed and stage of lactation (Mepham, 1987).

Mastitis

Bovine mastitis, defined as "inflammation of the mammary gland", can have an infectious or non-infectious aetiology (Bradley, 2002). Infectious mastitis results from the introduction and multiplication of pathogenic microorganisms in the mammary gland and this leads to a reduced synthetic activity, changes in the milk composition, and elevated milk somatic cell count (SCC) (Harmon, 1994).

Mastitis can be classified (Griffin, Morant & Dodd, 1987) according to its duration; acute (recent debut) or chronic (debut long time ago), or according to the presence or absence of clinical signs; clinical or subclinical. In clinical mastitis, there is one or more visible inflammatory signs present in the udder or in the milk, the milk composition may be abnormal and the SCC is increased. During a subclinical mastitis, the udder and milk shows no visible sign of inflammation but the milk composition is altered, specially the lactose content is decreased, while the SCC is increased (Bramley, 1992).

Suckling

Suckling is defined by Hall, Hudson & Brake (1988) as the behaviour of the young contributing to the procurement of milk from a nipple or teat. This behaviour includes locating and recognizing a potential provider of milk- usually the mother, searching for her nipples, recognizing and attaching to them, and then responding so as to facilitate milk withdrawal.

The neonate's first movements, after standing, are generally directed toward the mother and it finds a teat with seemingly random searching and nuzzling movements (Hafez & Bouissou, 1962). In beef cattle, newborn calves begin to suckle around 90 min after birth (Lidfors & Jensen, 1988).

It should be recognized that suckling and associated mother-offspring interactions may also play an important part in other aspects of development and production. For example, it has been observed that the release of oxytocin is greater in response to the suckling stimulus in cows compared to milking. In calves the feed intake related oxytocin levels are greater during suckling compared to when the calves are bucket fed (Lupoli *et al*., 2001). Oxytocin is required to obtain a proper milk ejection and disturbed milk ejection can cause decreased milk production (Bruckmaier & Blum, 1998). In addition, oxytocin stimulates maternal interaction and bonding between mother and young (Uvnäs-Moberg *et al*., 2001) and can stimulate weight gain, as shown in rats (Björkstrand & Uvnäs-Moberg, 1996). Furthermore, the welfare of the animals might possibly be improved since oxytocin has anti-stress effects (Uvnäs-Moberg, 1997) and in women is suggested to exert a short-term suppression of the cortisol response to mental stress (Heinrichs *et al*., 2001).

Another hormone which is secreted in greater amounts in response to suckling in cows is prolactin (Lupoli *et al*., 2001); this hormone is positively correlated with milk production (Koprowski & Tucker, 1973). It has been shown that cows suckled during 6 weeks after calving produce more milk (Bar-Peled *et al*., 1995). Because differentiation of mammary epithelium continues into early lactation in cattle, factors regulating secretion of prolactin during this period may influence mammary development and consequently milk production (Akers & Lefcourt, 1984).

Multiple suckling during early lactation has been reported to stimulate post-weaning milk production (Everitt & Phillips, 1971; Peel, Robinson & McGowan, 1979) and stimulate the butterfat production (Kaiser, 1975). In addition, milk production during first lactation tended to be higher for heifers that had been allowed to suckle milk as calves during the first 42 days of age than calves that had been fed with milk replacer (Bar-Peled *et al*., 1997). Furthermore, restricted suckling plus machine milking have been shown to reduce milk somatic cell count when compared to machine milking alone in tropical dairy cows (Margerison, Preston & Phillips, 2002).

Weaning and separation

Weaning is a natural stage of the reproductive and developmental process of the animals. During the weaning period the developing organism must make the major transition from a state of complete dependence on maternal care to one of independence switching from mothers milk to solid food as a source of nutrition (Martin, 1984). For most mammals, weaning is gradual and involves a progressive reduction in the rate of milk transfer from mother to young, accompanied by an increasing intake of solid food by the offspring and profound behavioural changes in the parent-offspring relationship (Martin, 1984).

In organic dairy farms where foster cows are used to rear alien calves, the weaning involves the abrupt end of suckling of the foster cow and absolute removal of the calves. This abrupt cow-calves separation is done in order to facilitate the farm management. Abrupt weaning not only disrupts the maternal bond between the calf and its dam, but also the social bond between the animal and their familiar social group which is an inherent aspect of weaning (Hickey, Drennan & Earley, 2003).

The cow-calf separation has been reported to be stressful to cows and calves and it seems that the longer the animals are allowed to stay together the stronger the behavioural response to separation is (Lidfors, 1996; Weary & Chua, 2000; Stehulova, Lidfors & Spinka, 2003). Furthermore, the calf presence and its removal can negatively influence oxytocin secretion during milking (Tancin *et al*., 2001).

Stress physiology

There are many definitions of stress throughout the scientific literature. One recent definition of stress by Pacak & Palkovits (2001) states that it is a state of physical or perceived threat to homeostasis. During stress, an adaptive compensatory specific response, by activation of specific central circuits, of the organism is activated in order to sustain homeostasis. In animal welfare studies, a more applied definition of stress is given by Dobson & Smith (2000) where stress is defined as the inability of an animal to cope with its environment. This phenomenon is revealed by a failure to achieve genetic potential, *e.g.* for growth rate, milk yield, disease resistance, or fertility.

The maintenance of homeostasis requires precise coordination of autonomic, neuroendocrine, and behavioural responses to contend with constant perturbations of the internal and external environments. The hypothalamic-pituitary-adrenal (HPA) axis is the most representative and probably the most effective neuroendocrine regulatory pathway in the stress response (Pacak & Palkovits, 2001).

The HPA axis can be activated in response to different stressors, for example isolation in unfamiliar surroundings (Veissier & Le Neindre, 1988; Boissy & Le Neindre, 1997; Rushen *et al*., 1999), transport (Zavy *et al*., 1992), handling (Solano *et al*., 2004) and dehorning (Mellor *et al*., 2002). The HPA axis activation is mediated by the hypothalamic paraventricular nucleus which releases corticotrophin releasing hormone and arginine vasopressin into the hypophyseal portal system to release ACTH from the pituitary gland (Herman & Cullinan, 1997; Dobson *et al*., 2003). ACTH stimulates the synthesis and release of glucocorticoids (cortisol and corticosterone) from the adrenal cortex. A recent study suggests that the endothelins (ET), a regulatory peptide produced by the adrenal cortex, may also play an important role in the regulation of corticosteroid secretion through activation of receptors in the adrenal gland (ET_A and ET_B) stimulating glucocorticoid secretion (Delarue *et al*., 2004).

In most mammals, cortisol is the primary glucocorticoid secreted by the zona fasciculata of the adrenal cortex and it is released within minutes after the exposure to a stressful situation. The main function of cortisol is to mobilize energy reserves to promote hyperglycemia by stimulating hepatic gluconeogenesis and reducing cellular glucose uptake (Borski, 2000). Under acute stress situations, cortisol allows an individual to respond to stressors by supporting energy mobilization (Erickson, Drevets & Schulkin, 2003) and mediating the re-distribution of lymphocytes and macrophages to the sites of acute challenge (McEwen, 1998). However, chronic elevation of cortisol results in protein catabolism (Moberg & Mench, 2001), suppression of the immune activation of circulating leukocytes, inhibition of cytokines production and other mediators of inflammation, thus increasing susceptibility to infectious agents (Chrousos, 1995; Sapolsky, Romero & Munck, 2000).

The cortisol secretion in cattle exhibits circadian rhythms with peak values in the morning (Thun *et al*., 1981) and strong ultradian rhythms with periods around 120 min. (Lefcourt *et al*., 1993). This is important to consider when assessing cortisol response of animals to stressors. In pigs it has been shown that the timing of the stressor is important for the acute cortisol response, resulting in a greater cortisol increase in response to stress in the morning (Ruis *et al*., 1997).

Cortisol in plasma and saliva

The assessment of cortisol in saliva has gained interest in studies evaluating the HPA axis activation in different species including humans (Aardal-Eriksson, Karlberg & Holm, 1998), cattle (Fell, Wells & Shutt, 1986), sheep (Fell, Shutt & Bentley, 1985), goats (Greenwood & Shutt, 1992), pigs (Parrott, Misson & Baldwin, 1989), dogs (Cronin *et al*., 2003), and horses (Shanahan, 2003). Measuring cortisol responses in saliva instead of plasma or serum samples has certain advantages. For example, taking saliva samples is a relatively stress free procedure, does not require trained personnel and the saliva samples can be stored at 20°C for up to 4 weeks without significant reduction in cortisol levels (Kirschbaum & Hellhammer, 1989; Kirschbaum & Hellhammer, 1994). Furthermore, the cortisol concentrations in saliva are representative of the unbound (free) fraction of the hormone in serum (Lac, 2001) and only this unbound cortisol is able to reach target tissues and elicit its glucocorticoid effect (Kirschbaum & Hellhammer, 1994). The assessment of salivary cortisol has been suggested to be a more appropriate measure of adrenocortical responses than cortisol determination in blood samples (Vining *et al*., 1983; Cook *et al*., 1996).

A part of cortisol is converted into cortisone by 11β-Hydroxysteroid dehydrogenase (11βHSD) in the salivary glands, but this does not modify significantly the validity of the cortisol assay (Lac, 2001). 11βHSD is an enzyme system that catalyses the interconversion of active glucocorticoids to their inactive metabolites and is now established as a crucial mechanism modulating corticosteroid hormone action (Penning, 1997; Walker & Stewart, 2003). Two isoforms of the enzyme 11βHSD have been identified, 11βHSD1 which acts predominantly as an NADP(H)-dependent reductase to generate active cortisol, whereas 11βHSD2 acts exclusively as an NAD^+-dependent enzyme that catalyses the enzymatic inactivation of cortisol to cortisone (Michael, Thurston & Rae, 2003).

Introduction to the research reports

Mastitis is the most economically important disease of dairy cattle, accounting for 38% of the total direct costs of the common production diseases (Kossaibati & Esslemont, 1997). The losses associated with mastitis arise from the costs of treatment, culling, death, decreased milk production (Bradley, 2002; Grohn *et al*., 2004) and price penalties for milk with a high somatic cell count (SCC) (Hemingway, 1999).

In organic dairy farms, one way of dealing with the cows that have high milk SCC or mastitis is to use them as a foster cow. These cows are usually kept with the calves allowing them to suckle for a number of weeks. The number of calves suckling each cow is based on the milk yield of the foster cow. The statutes for organic dairy farms in Sweden (KRAV, 2004), states that the calves should drink whole milk by suckling, either from a cow or from an artificial teat for at least 12 weeks after birth. Therefore, having a foster cow nursing three to four calves eliminates the workload of milking the cows and later on feeding the calves. This also gives the farmer the opportunity to use the milk from cows with a high milk SCC for feeding the calves.

In addition, some other advantages of having the cows nursing calves have been reported previously, for example reduced risk of mastitis (Everitt, Phillips & Whiteman, 1968) and reduced milk SCC (Margerison, Preston & Phillips, 2002). However, when the suckling period is terminated the procedure of weaning and separating the animals is often a stressful procedure for both cows and calves and might have some negative effects on the production and welfare of the animals. Some examples of this negative effect are decreased weight gain in calves, increased levels of cortisol in cows and calves (that in the long run can compromise the immunity system of the animals) and inhibition of the milk ejection.

For these reasons, the aim of the first study was to evaluate the effects of nursing four calves during 9 weeks and the subsequent weaning of cows with previous high SCC on their saleable milk yield (total milk minus discarded milk due to high CMT score), milk composition, udder health and behaviour during milking.

The assessment of cortisol in saliva has been reported to be a reliable method to assess the activation of the HPA axis in response to stress in several species including humans, dogs, pigs and horses. However, to the knowledge of the author there are only two studies in cattle that describe the relationship between values of cortisol in plasma and saliva. These studies report different results, one shows a good correlation between values of blood and saliva after ACTH stimulation in calves and after machine milking in cows (Negrao et al., 2004). The other, reports significant correlations only before ACTH stimulation in calves but not after it (Steinhardt & Thielscher, 2000). In addition, a time lag between values of cortisol in plasma and saliva has been reported in sheep (Cook, 2002).

For these reasons the aim of the second study was to further characterize the relationship between salivary and plasma cortisol in response to stress in cattle.

These studies are part of a larger project, which evaluates the effects of two different methods of weaning and separation of four calves from a foster cow. The two different methods used were A) weaning and separation in one-step (at 10 weeks of age) and B) weaning and separation in two steps (weaning at 10 weeks and separation at 12 weeks of age).

Aims of the investigation

The general aim of the present studies was to evaluate the effects of suckling on the milk composition, milk yield and udder health as well as the stress response to weaning and separation in cows and calves.

Specific aims

- To evaluate the effects of nursing four calves for 9 weeks and subsequent weaning on the saleable milk yield, milk composition, udder health and behaviour during milking in cows with previous high SCC or mastitis.

- To characterize the relationship between salivary and plasma cortisol response to stress in cows and calves.

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