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Mastitis in Sows

Clinical, bacteriological and cytological examinations in assessing udder health during early lactation and at weaning

Arne Persson

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Av fakultetsnämnden utsedd opponent: Professor Lennart Bäckström, University of Wisconsin, School of Veterinary Medicine, Department of Medical Sciences, Madison, WI 53706, USA.

Abstract

Mastitis during the early lactation in sows is predominantly caused by *Escherichia coli*, and implicated in the agalactia post partum (APP). Chronic granulomatous mastitis (CGM) has a polybacterial etiology often diagnosed at time of weaning. *E. coli* mastitis in APP sows and the CGM in lactating sows lead to impaired udder health. The first study was designed to obtain more information about the potential influence of 2 late gestation feeding regimes (restricted vs standard) on health status of sows with reference to APP. The second study focused on teat injuries and palpable changes of the mammary glands and their potential impact on udder health at time of weaning. The third study comprised CGM sows that were examined before and after slaughter. In all three studies, results from thorough clinical examinations of the sows and their udders were confirmed by bacteriological and cytological analyses of milk amplified with analyses of pH during the early lactation period and histopathological examinations of mammary tissue specimens after weaning.

The incidence of APP was significantly lower (14.4%) in the restricted-fed compared with the standard-fed sows (26.6%). APP sows showed a significant increase in rectal temperature one day before parturition and an increase in number of stillborn piglets (0.4) compared with clinically healthy sows, indicating an establishment of the disease before partus.

Bacteriological examination of colostrum from APP sows and from clinically healthy sows, yielded growth of *E. coli* from one or several glands in 80% and 30% of the lactations, respectively. The growth of *E. coli* declined rapidly and was eliminated between days 3 and 8. Healthy sows with *E. coli* were designated as being subclinically infected. In colostrum, a substantial elevation of the total cell content (TCC) and its percentage of

polymorphonuclear leucocytes (PMNLs) was demonstrated in 49 (64%) out of 77 APP lactations and 15 (16%) out of 96 clinically healthy lactations. Udder symptoms of clinical mastitis were poorly correlated to *E. coli* mastitis (TCC>$10x10^6$).

Teat and udder skin injuries were numerically more frequent within the milk-producing glands of the udder. The prevalence of teat injuries subsided and 76% of the teat injuries were clinically normal at re-examination 7 days post weaning. The prevalence of palpable changes increased from first to second and subsequent lactations (15%, 30% and 60%).

CGM was confirmed by histopathological examination in 10 out of 11 sows. A polybacterial flora predominated and comprised of *S. aureus*, *A. pyogenes*, *F. necrophorum*, *P. anaerobicus*, *P. granulosum* and *Prevotella* spp. As an appendant result, a 'new' bacteria belonging to the genus *Actinomyces* (Actinomyces - EF group 78, CCUG 37626) was isolated from tissue collected from an additional sow with CGM.

Key words: agalactia post partum, feeding, *Escherichia coli* mastitis, colostrum, milk, cytology, bacteriology, pH, weaning, teat injuries, chronic granulomatous mastitis

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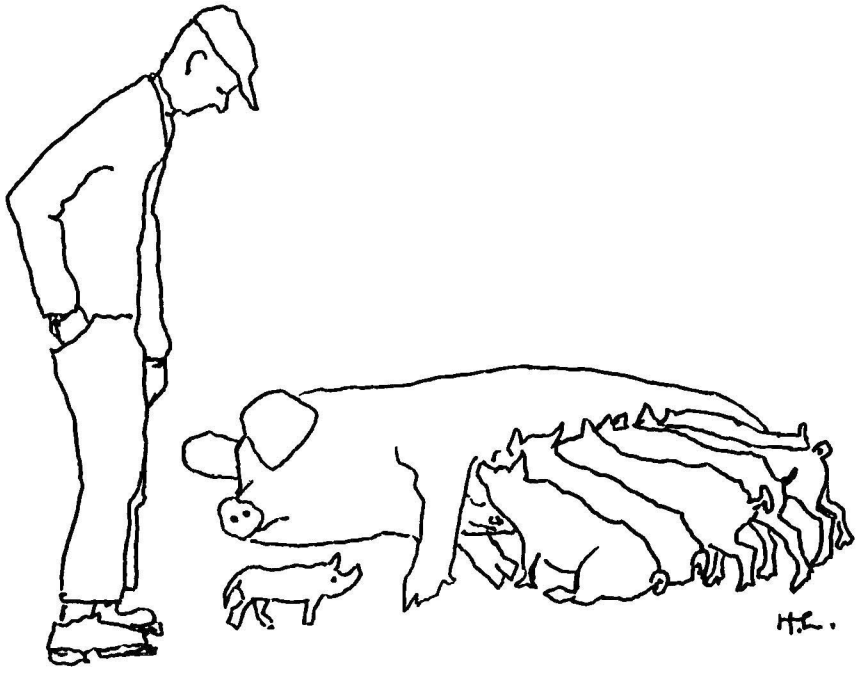

To my family
Maria
Martin and Kristina

Abstract

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Contents

Appendix

Papers I-V

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

I. Persson, A., Pedersen, A-E., Göransson, L. & Kuhl, W., 1989. A long term study on the health status and performance of sows on different feed allowances during late pregnancy I. Clinical observations, with special reference to agalactia post partum.

II. Persson, A., Pedersen-Mörner, A. & Kuhl, W., 1996. A long term study on the health status and performance of sows on different feed allowances during late pregnancy II. The total cell content and its percentage of polymorphonuclear leucocytes in pathogen-free colostrum and milk collected from clinically healthy sows.

III. Persson, A., Pedersen-Mörner, A. & Kuhl, W., 1996. A long term study on the health status and performance of sows on different feed allowances during late pregnancy III. *Eschericha coli* and other bacteria, total cell content, polymorphonuclear leucocytes and pH in colostrum and milk during the first 3 weeks of lactation.

IV. Persson, A., 1997. Clinical assessment of udder health status of sows at time of weaning with special reference to bacteriology and cytology in milk. J. Vet. Med. A 44, 143-158.

V. Persson, A., Karlstam, E. & Jonsson P., 1997. Chronic granulomatous mastitis in sows with special reference to bacteriology, cytology and histopathology. Manuscript.

Introduction

Background

Maintenance of a proper health status of the sow in general and of udder health in particular is necessary to enable her to produce a sufficient amount of milk throughout the lactation period. The mastitis implicated in the agalactia post partum (APP) syndrome often causes a transient lactation failure during the post partum period. The disease may also cause a partial or, in severe cases, total cessation of milk production, causing starvation and a subsequent increase in piglet mortality (Ringarp, 1960; Striegel & Kunesh, 1974; Jorsal, 1983). The incidence and importance of the APP syndrome have varied during the last 30-40 years. In a comprehensive field study carried out in south-west of Sweden, 1957-1958, Ringarp (1960) reported an incidence of 'agalactia toxaemica' of 3.7%. In a later Swedish study, the incidence was reported to vary from 5.5% in small herds to 10.3% in large herds (Bäckström, 1973). During the same period, the mastitis-metritis-agalactia (MMA) syndrome was listed as the number one swine health problem in the USA (Striegel & Kunesh, 1974). In Sweden, the problem with the APP syndrome probably culminated during the 1970s, when the incidence of APP could exceed 50% in severely affected herds (Sandstedt et al., 1979). In Denmark, a survey over a 4-year period revealed that 9.6% of culled sows were dispatched because they had suffered from mastitis, metritis and/or agalactia (Svendsen et al., 1975). In more recent comprehensive field studies the incidence of APP was 6.9% in the USA (Bäckström et al., 1984), 9.5% in Denmark (Jorsal, 1983) and 17.5% in Norway (Lingaas & Rønningen, 1991).

During the remaining lactation, the competition between piglets might be one of several factors that damage the mammary glands and teats, predisposing to infectious disturbances during lactation or during the involution period post weaning. The pathological transformation of the mammary glands leads to a permanent decrease in lactation potential. The frequencies of teat- and udder skin injuries during the lactation period or at the time of weaning have only been studied to a limited extent (Svendsen et al., 1984; Edwards & Lightfoot, 1986; Bollwahn & Meermeier, 1989; Hultén et al., 1995). Teat wounds might be infected by ubiquitous bacteria and lead to inflammation, fibrosis and occlusion of the teat canals (Done, 1980). Further, the ubiquitous bacteria might also invade the mammary glands during the lactation period or at the time of weaning, causing chronic mastitis (Delgado & Jones, 1981; Bollwahn & Meermeier, 1989) with successively impaired udder health status and, as a consequence, predispose to malnutrition of the offspring (Englert et al., 1956). In an abattoir survey, the frequencies of mammary abscesses varied from 18.5% of nonlactating sows to 20.4% of lactating sows (Delgado & Jones, 1981). In a recent study (1995), Jonsson found that 2.6% of Swedish Landrace sows and 2.1% of Swedish Yorkshire sows were dispatched from hybrid-producing herds because of mammary abscesses. Veterinary practitioners claim that the problem is increasing in sow herds in Sweden (Kugelberg & Sjögren, 1994, pers. comm.).

The mammary glands and teats, lactation and sucking behaviour

The mammary glands of the sow consist of 2 thoracic pairs, 4 abdominal pairs and one inguinal pair suspended in two parallel rows along the ventral abdominal wall. The cylindrical mammary papillae (teats) are between 2 and 3.5 cm long, depending on whether the mammary glands are lactating or not. Each teat has two canals and orifices which terminate two separate cavity systems, one anterior and one posterior (Fig. 1). In exceptional cases, three teat canals are present, of which one often ends blindly at the base of the teat. The teat canal is 3 to 4 mm long and is not distinctly separated from the teat cisterna. The teat cisterna continue further without distinct demarcation into the gland cisterna (for complete and more detailed information, see Schummer et al., 1981).

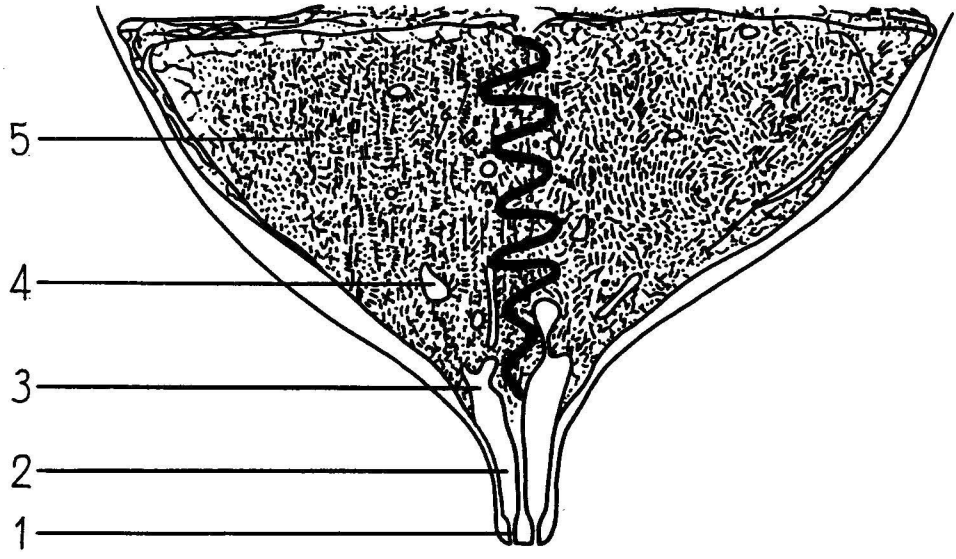

Figure 1. Sagittal section through one mammary gland of the udder of a sow. The wavy black line separates a front and a rear segment. 1. teat canal; 2. teat sinus; 3. gland sinus; 4. lactiferous duct; 5. parenchyma of the gland.

The daily milk production varies as a function of litter size, which means that milk production increases parallel with an increase in litter size, although in a large litter each piglet receives less milk per day compared with piglets in a small litter (Salmon-Legagneur, 1965; cited Smith et al., 1992). The milk production reaches a peak during the third and fourth weeks of lactation (Allen & Lasley, 1960).

Milk ejection occurs in the sow every 45 min. with over 20% of the suckling periods ending as a failure (Ellendorff & Poulain, 1984). The nursing and suck-ling last for 2-3 min., while the actual flow of milk is confined to 10 or 20 seconds (for review, see Fraser, 1980). The piglets show a preference for the more anterior, teats with about 90% occupancy of teats in the first three pairs, declining to 30% at the seventh pair (Fraser & Thompson, 1991). Non-suckled glands and teats cease to produce milk already 1- 2 days after farrowing (Dellmeier & Friend, 1991).

Involution of the mammary gland

The involution of the mammary gland tissue is generally more rapid in sows than in cows (Schultz et al., 1977). According to Salomé Rahm et al. (1984), the involution is macroscopically characterized by a distension of the mammary glands which lasts for 24-48 hours post weaning (p.w). On day 4 p.w. it is not possible to obtain any secretion and the skin of the gland starts wrinkling. One week p.w., the mammary glands have been lifted up towards the abdominal wall. The involution of the glandular tissue has been histologically divided into 4 separate phases: a/ milk accumulation during the first 2-3 days, b/ breakdown and reconstruction changes on days 3-9, c/ slow reconstruction on days 9-28 and d/ resting phase from the 28th day.

Cytology and pH in colostrum and milk

The total cell content or the somatic cell content is a useful and reliable parameter monitoring udder health status in lactating cows. Also in sows the total cell content (TCC) per ml together with the percentage of polymorphonuclear leucocytes (PMNLs) in colostrum or milk have been demonstrated to reflect the health status of the mammary glands.

The levels of TCC and PMNLs in sow colostrum and sow milk have been estimated in a few studies (Table 1). In a study carried out by Wegmann (1985), milk samples were frequently collected throughout a lactation period of 30 days with the aim of obtaining information on the cell count as well as the percentage of granulocytes in colostrum and milk from normal as well as from mastitic mammary glands. In other studies, the samplings were more infrequent and (or) the numbers of sampled sows low. Evans et al. (1982) focused on cell morphology and the cell count levels were used as supporting information. In a recent study by Drendel (1991), the pH, the concentration of lysozyme and the conductivity in sow milk were related to the total cell count. When comparing the results of the cell counts from earlier investigations (Table 1), it is likely that the different objectives of the studies and the heterogeneous groups of sows have substantially contributed to the variation in milk cell content.

The physical properties of colostrum and milk in sows might be monitored by, e.g. pH, as in cows. During the first three days post partum, the pH was measured to values ranging from 6.4 - 6.5 (Ringarp, 1960), 6.2 - 6.9 (average c. 6.5; Plonait, 1961) or 6.0 - 6.9 (average 6.6; Martin et al., 1967) in normal lactating sows. The remaining lactation period is characterized by a gradual increase in pH, reaching 7.0 at day 40 (Plonait, 1961). The result from a recent study by Drendel (1991), where the pH was measured during a lactation period of 35 days, revealed pH-levels similar to those described above.

Analyses of the cell content and pH in colostrum and milk might be useful tools in experimental studies to monitor the udder health status in sows during different phases of the lactation. The use of TCC, PMNLs and pH as indicators of mastitic reactions requires detailed knowledge of the normal cell content as well as the pH in colostrum and milk. This could be achieved by analysing milk secretions from phases of lactation, including the time of weaning.

Table 1. Studies on the total cell content [TCC] ($x10^{-6}$) and the polymorphonuclear leucocytes [PMNLs] (% / absolute numbers[6]) in colostrum and milk from sows

Day of lactation	References													
	Evans et al.[1] 1982		Weber and Ferguson[2] 1982		Wegmann 1985		Schollenberger et al.[1,3] 1986		Hurley and Grieve[4] 1988		Magnusson et al.[5] 1991		Drendel 1991	
	TCC	PMNL%	TCC	PMNL%	TCC	PMNL%	TCC	PMNL%	TCC	PMNL%	TCC	PMNL%	TCC	PMNL[6]
0-1	10.0	71.7	0.57	80.7	0.3-0.7	<10				55.5	9.0 (A)	58.0 (A)	1.88	0.90
							1.1	61.2			5.1 (P)	65.5 (P)		
2					0.5-0.9	<10								
3		55.4	4.48	86.3	0.9-1.1	<10				30.3			2.42	1.13
4					1.7	<10								
5			6.31	86.0	0.9	<10	1.4	50.9					2.93	1.41
6					0.5	<10							2.47	1.12
7									0.25 (min.)	25.6	4.5 (A) 2.4 (P)	13.9 (A) 20.9 (P)		
8			5.43	83.5	0.5	<10								
10	1.0	39.2	5.81	89.4										
11					0.6	<10	2.1	41.5						
12			5.88	91.3										
14										11.7	4.8 (A) 5.2 (P)	18.4 (A) 7.6 (P)		
15		51.3	2.57	90.8	0.6	<10								
17			3.56	79.7										
18					1.3	<10								
19			6.41	64.3			1.1	43.9						
20		32.1												
21									0.75 (max.)	13.9			2.65	1.34
22			2.32	90.8	1.2	<10								
25					2.4	<10								
28							1.5	50.4		43.5	1.5 (A) 4.2 (P)	33.0 (A) 14.2 (P)	2.02	1.25
29 - 35					1.0	<10							2.45	0.96

1. PMNLs comprise only neutrophils. **2.** Results only available from IPVS-proc., Mexico City, 1982. **3.** Average TCC- and PMNL-values for each period, denoted with brackets. **4.** Min. (d.7) and max. (d.21) TCC within the lactation period of 28 d. **5.** Abbreviations: A and P = anterior and posterior mammary glands, respectively.

Agalactia post partum (APP)

Clinical symptoms

The 'classical' APP syndrome appears within the first 12-48 hours post partum and the clinical symptoms are predominated by elevated rectal temperature (>39.5°C) and unwillingness to eat and drink; the total impact of the disease leading to a lethargic general condition of the sow (Ringarp, 1960; Martin et al., 1967; Hermansson et al., 1978a). A crucial problem arises when establishing a threshold above which the rectal temperature indicates a disease condition. Another important observation is that the rectal temperature of sows increases at parturition, the rise being 0.7°C to 1.0°C (King et al., 1972; Hendrix et al., 1978; Elmore et al., 1979). Nevertheless, determination of the rectal temperature is of great importance since it provides us with objective clinical data. Observation of the rectal temperature prepartum might indicate early stages of the disease, preceding subsequent impaired clinical health of the sow post partum.

Although most diseased sows often are in lateral recumbency, sows with clinical signs of mastitis are in sternal recumbency in attempts to protect the udder (Hermansson et al., 1978a). Although the piglets try to massage the udder with their snouts the sow persists in her reluctance to nurse. The unwillingness of the sow to nurse her piglets, together with a decrease in milk production - hypogalactia - (Ross et al., 1975), rather rapidly results in the piglets becoming agitated. If the general condition of the sow does not become restored, the piglets lose weight. The sows affected by the APP syndrome will, on average, wean 0.4 - 0.5 piglets less than healthy sows (Hermansson et al., 1978a; Jorsal, 1983). The clinical symptoms of mastitis are characterized as swelling and hardening of one or more mammary glands which, in more severe cases, is combined with reddening and (or) soreness. The physiological swelling that occurs after farrowing might be difficult to distinguish from a pathological swelling caused by inflammation of the mammary glands. In a field study where agalactic sows were compared with healthy controls within the same herd, clinical examination of the udders of diseased sows revealed mastitis in 50% (Hermansson et al., 1978a).

Vaginal discharge recorded post partum seems to be as frequent in healthy sows as in sows affected with APP (Hermansson et al., 1978a). From a general point of view, metritis or endometritis has been considered as a minor etiological factor implicated in the disease syndrome (Ringarp, 1960; Martin et al., 1967; Nachreiner et al., 1971).

Bacteriology, pathogenesis and milk cell content

Gram-negative bacteria such as *Escherichia coli* (Ringarp, 1960; Martin et al., 1967; Armstrong et al., 1968; Thurman & Simon, 1970; Bertschinger et al., 1977; Middleton-Williams et al., 1977; Ross et al., 1981) and to some extent *Klebsiella* spp. (Lake & Jones, 1970; Done, 1975; Ross et al., 1975; Bertschinger at al., 1977) seem to be strongly implicated in the etiology causing mastitis in sows during the immediate post partum period. *E. coli* has been isolated from clinically normal as well as mastitic mammary glands (Ross et al., 1981). Wegmann (1985)

reported that the number of *E. coli* bacteria that had invaded the mammary glands declined in number, with a final elimination of the bacteria within 5 days post partum. The different serotypes of *E. coli*, all show a large heterogeneity (Armstrong et al., 1968; Bertschinger et al., 1977), which is in accordance with results from *E. coli* mastitis in cows (Eberhart et al., 1979; Eberhart, 1984).

Other bacteria such as *Staphylococcus* spp. and β-haemolytic streptococci have been isolated from tissue specimens of mammary glands, in most cases without causing any microscopic evidence of mastitis (Ross et al., 1981).

The hypogalactic, or in more severe cases agalactic, condition in sows seems to be mediated by the release of endotoxin from the gram-negative bacteria. These causes and effects have been indicated by clinical and haematological changes observed in field studies (e.g. Hermansson et al., 1978b), but also confirmed under experimental conditions (Nachreiner et al., 1972; Nachreiner & Ginther, 1974). Although only partly understood, the endotoxin exerts its effect in an intricate way upon the endocrine and immune functions. Prolactin, which is essential for maintenance of the milk production in the sow, is significantly lower in sows affected by APP when compared with clinically healthy sows from the same herd (Threlfall et al., 1974). Injection of *Escherichia coli* endotoxin at low dosages at day 2 post partum suppresses the prolactin (Wagner, 1982; Smith & Wagner, 1984), an effect that also might be indirectly mediated by interleukin-1 (de Ruijter et al., 1988). This suppression in the release of prolactin is not observed when the endotoxin is injected 6 days post partum (Smith & Wagner, 1985). The observed effects might be explained by a higher sensitivity of the hypothalamic-pituitary axis during lactogenesis. The suppression of prolactin leads to a decrease in milk production causing a transient reduction in piglet growth rate (Wagner, 1982).

Mammary glands of sows have been defined as mastitic if the TCC in the secretion is $>5.0 \times 10^6$ cells/ml and the percentage of PMNLs is ≥70 (Wegmann, 1985). This elevation in TCC also occurs when mastitis is caused by *E. coli* (Wegmann, 1985; Bertschinger et al., 1990). In mastitic glands, the TCC stabilizes after 10 days on levels between $2.4\text{-}3.1 \times 10^6$ cells/ml, while the PMNLs tend to decrease to levels below 70% already after 2 days (Wegmann, 1985).

The pH-values in colostrum and milk from sows suffering from 'agalactia toxaemica' are slightly higher, 7.0 - 7.2 (Ringarp, 1960) or 6.5-8.0 (average 7.2; Martin et al., 1967), compared with pH from clinically non-mastitic glands.

Monitoring the TCC, the PMNLs and the pH in bacteriologically negative and positive (predominated by *E. coli*) colostrum and milk collected from clinically healthy sows as well as from sows suffering from APP would increase our knowledge in this field substantially.

Influence of feeding

The hypothesis of a negative influence of improper feeding regimes imposed upon sows during late gestation was raised in Sweden already during the early 1950's (Sandstedt, 1953). Since then, the risk of overfeeding sows, including feeding excessive protein, has been of particular consideration (Ringarp, 1960; Sandstedt et al., 1979).

The result of a comprehensive Swedish study of the 'agalactia toxaemica' syndrome clearly indicates that an interrelationship exists between the syndrome and intoxications and (or) infections of gastrointestinal origin and that deficiencies in feed and feed-hygiene are predisposing factors of significance (Ringarp, 1960). Different feeding regimes, such as drastic dietary changes, feeding poor quality fodder or feeding the sows large quantities of skim milk (excess protein) during late gestation, have been found to produce clinical symptoms characteristic of the 'agalactia toxaemica' syndrome (Ringarp, 1960). However, confusion might arise when interpreting differences in the clinical symptoms observed and how the intensity of these symptoms varies between sows belonging to different herds (Ringarp, 1960).

The influence of a restricted feeding intensity during the final 3 weeks of the gestation period seems to cause a substantial decrease in the occurrence of MMA in sows (Sandstedt et al., 1979). Møller Jensen (1981) has shown that the implementation of a restricted feed allowance, as was suggested by Sandstedt et al. (1979), was found not only to decrease the incidence of MMA but also mitigate the intensity of the clinical symptoms in diseased sows. A reduction of the percentage of crude protein from 14-15% to 10-11% in the late gestation feed mixture also resulted in a substantial reduction in the expected incidence of MMA (Sandstedt et al., 1979). The use of laxative feed such as wheat bran, substituting half of the regular feed amount, has been suggested to be a successfully prophylactic treatment, as constipation is a common clinical component of the 'agalactia toxaemica' syndrome (Ringarp, 1960). The result from one study indicates that an increase in the crude fibre content of the diet might decrease the incidence of agalactia (Göransson, 1989a). In another recently published study, the influence of an all-vegetable protein diet upon the health status of sows post partum indicates a decrease in sows suffering from agalactia (Göransson, 1990b).

An intricate interrelationship seems to exist between deficiencies in the feeding regimes or feed-hygiene and the APP syndrome. It would therefore be of interest, under standardized conditions, to study the effects of a very restricted feed allowance during the late gestation period upon the health status and performance of sows.

Teat injuries and chronic mastitis

The piglets start to set up a relatively stable 'teat order' already within the first hours after birth (Hartsock & Graves, 1976), resulting in fights between littermates. This fighting continues up to one week of age (Newberry & Wood-Gush, 1985), but the competition continues to be important throughout lactation (Fraser, 1990). Teeth resection is performed by many farmers to minimize laceration of the faces of littermates but also in attempts to minimize skin injuries of the mammary glands of their mother (Fraser, 1975). Omission of the resection of the needle-teeth resulted in increased frequency of bites on the sow's udder (Hutter, 1993). The fighting might be one of several factors that damage the mammary glands and teats, thus predisposing for impaired udder health. Exposure of the mammary glands and teats to different flooring in the farrowing pens has

been investigated in some studies (Svendsen et al., 1984; Edwards & Lightfoot, 1986). The frequency of teat injuries and teat cuts was increased if a perforated floor was used and tended to be highest at weaning (Edwards & Lightfoot, 1986). The frequency of acute teat wounds among recently weaned sows was found to be 3.7 % in a survey of sows slaughtered at a commercial abattoir (Bollwahn & Meermeier, 1989).

Ubiquitous bacteria might colonize wounds of the mammary glands and teats in sows, mainly caused by penetration of the sharp teeth of the piglets (Magnusson, 1928; Englert, 1961; Renk, 1962; Jones, 1980; Meermeier, 1987), implying a risk of causing inflammation, fibrosis and occlusion of the teat canals (Done, 1980), and further invade the mammary glands, causing chronic mastitis with formation of abscesses, phlegmonas and granulomas. A heterogeneous microbiological flora comprising aerobic as well as anaerobic bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Actinomyces pyogenes*, *Bacteroides* spp., *Fusobacterium necrophorum* and *Pasteurella multocida* are frequently isolated concomitant with post mortem examination of cases of chronic mastitis (Delgado & Jones, 1981; Bollwahn & Meermeier, 1989). *Actinomyces suis* is another bacteria that has been suggested to be of major importance as an etiological agent of the disease (Englert, 1961; Franke, 1973a,b). In a recent study, Franke received support from Oomi et al. (1994). Patchimasiri et al. (1994) claim that *A. suis* can be isolated from 10% of examined tonsils of 1-year-old pigs. Further, it is suggested that sucking pigs will transmit *A. suis* from their mouths through incisions or injuries of the mammary glands (Oomi et al., 1994). The ultimate consequence of these chronic types of mastitis might be the formation of what is clinically denoted 'actinomycosis', which in pathological terminology is determined as chronic granulomatous mastitis. The morphological appearance of this chronic mastitis is a moderately enlarged mammary gland, which in exceptional cases reaches the size of large tumours - actinomycomas - hanging down from the udder (Magnusson, 1928). The pyogenic centre of the granulomas often fistulate and infectious material is spread through open ulcers to the environment (Eich, 1984). The normal mammary tissue is successively destroyed and replaced with a dense connective tissue. The general condition of the sows is not disturbed and the piglets are allowed to massage and suckle the mammary glands (Eich, 1984). The impaired udder health status of the sow leads to decreased milk production and thus predisposes for malnutrition of the offspring (Englert et al., 1956).

Available information is very limited in the area of teat injuries caused by external factors. Since perforated floors are generally not used in Swedish farrowing pens, it might be assumed that the sucking of the pigs might be one factor that exerts a more pronounced effect when contributing to the etiology of teat injuries. It would therefore be of first priority to submit the prevalence of teat injuries at weaning under normal herd conditions to unbiased examination. Veterinary practitioners claim that udder health problems with special emphasis on chronic mastitis are problem on the increase. Since no extensive study has been carried out in Sweden for many decades, it might be of highest priority to start to

investigate the problem from an approach where clinical, microbiological and pathological examinations are combined.

Aims of the study

The aims of the present work on sows were to study:

- the effects of a restricted feed level versus a standard feed level during late pregnancy upon the clinical health status and performance of the sows, with special reference to the agalactia post partum (APP);

- the TCC, its proportion of PMNLs, and pH in bacteria-free colostrum and milk, on days 1, 3, 8 and 22 of lactation in 6 consecutive lactations, and the potential influence of the two feeding regimes;

- the TCC, its proportion of PMNLs, and pH in bacteriologically negative or *E. coli* positive colostrum and milk from APP sows and from clinically healthy sows;

- the mammary glands and teats of sows at weaning and at 7 days after weaning with special focus on teat injuries and palpatory changes of mammary tissue;

- the bacteriology, the TCC and its proportion of PMNLs in milk/secretion from normal and from clinically abnormal mammary glands and teats at weaning and at 7 days after weaning;

- the bacteriology, milk-cytology and histopathology with reference to clinically diagnosed chronic granulomatous mastitis (CGM).

Material and methods

Animals and locations

The sows included in the experiments were 39 pairs of full sibs (Papers I, II and III). They were cross-bred Swedish Landrace x Swedish Yorkshire. Twenty-four of the 39 pairs entered the trial as gilts and were kept for 6 parities if possible. One sow in each pair was randomly allocated to the control group, and the other to the experimental group. The investigation was carried out at the Research Station at Funbo-Lövsta, Department of Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden. The results of this study were obtained from summer 1981 to autumn 1984.

The animals in paper IV comprised 76 sows (mainly Swedish Landrace x Swedish Yorkshire) belonging to three commercial pig farms, supervised by the Swine Herd Health Practice of the Ambulatory Clinic (Department of Obstetrics and Gynaecology, Swedish University of Agricultural Sciences). The investigation was carried out during spring and autumn 1994. The sows were farrowing in batches of 15 to 20. Twenty-five sows were examined after two consecutive lactations.

The 11 sows in paper V (mainly Swedish Landrace x Swedish Yorkshire) belonged to six commercial pig farms, where the frequency of sows affected by chronic mastitis tended to increase. Six of the sows examined emanated from three farms, that obtained their pregnant sows from a 'sow-pool'.

In the chapter summarizing the results of this thesis (p. 34), another three sows from one farm are included. On this particular farm, the annual culling frequencies during 1995 and 1996 were estimated to 50%, mainly caused by disturbed udder health predominated by chronic mastitis.

Rearing systems

Three weeks before expected farrowing, the sows were transferred to thoroughly cleaned solid-floor pens with separate and solid dunging area (Papers I, II and III). All sows were kept loose during farrowing and lactation. The piglets were weaned at 6 weeks of age. At weaning, the sows were moved to stalls next to the boar for mating. After mating, the sows were kept in pens in groups of 4 but were individually fed.

The sows in paper IV were moved to individual pens before farrowing. The sows were allowed to move around during farrowing and lactation. The sows had access to separate dunging areas in two of three farms. In a few cases, the litters were subjected to cross-fostering within the first few days post partum. In paper V, the accommodations for the sows during lactation were similar to those described in paper IV, with the exception of one farm where each farrowing pen was occupied by three sows and their respective litters, freely moving around during the lactation period.

Diets and feeding regimes

In the first experiment (Papers I, II and III), the sows were fed a commercial cereal-based type of diet with 11.5 MJ/kg per kg and 14.9% crude protein. All animals had free access to straw and water. The animals in the control group (C) were fed 2.4 kg per day during the first 100 days of pregnancy and then 3.4 kg per day until farrowing. The experimental group (E) was offered extra between 30-100 days of gestation and then 1.0 kg daily (minerals and vitamins supplemented) during the last 15 days of gestation. The total amount of feed given during the gestation period was equalized for both groups. During lactation, the daily feed allowances were successively increased for all sows to a maximum of 4.0 kg + 0.2 per piglet within 14 days after farrowing.

In paper IV, the sows were fed commercial concentrates mixed preferably with barley and to a minor degree with oats, wheat or rye wheat. The levels of energy and digestible crude protein per kilogram were 12.6 MJ and 145 g, respectively, approximately following Swedish sow diet standards (Simonsson, 1993). The sows were offered 6-10 kg per day during lactation (adjusted to number of piglets per litter) and 4 kg per day after weaning until breeding and thereafter approximately 2.5 kg per day. The sows included in paper V were also fed according to Swedish stock-breeding standards.

Clinical examination and collection of colostrum and milk

The rectal temperature of the sows was monitored every morning and evening, starting 2 days before expected farrowing and continuing until 2 days after parturition (Papers I, II and III). Sows with a temperature exceeding 39.5°C within 48 hours after parturition were considered to be suffering from agalactia post partum (APP) and were clinically examined by a veterinarian. The general condition and temperament of the sow, appetite and thirst were recorded and the external genital organs were inspected.

Mammary glands with signs of inflammation such as swelling, reddening or soreness were selected for milk sampling together with clinically normal glands opposite to the affected ones. The piglets were removed from the sow 30 minutes before milking. The udder was thoroughly washed with soap solution and warm water followed by careful disinfection with iodine and 70% alcohol. Oxytocin (20 IU) was given i.m. to promote milk let-down. The first streams of secretion were discarded from both canals of the teat. Approximately 0.5 ml was collected for bacteriological and 2 ml for cytological analysis, each of the samples in separate plastic tubes. Milk from both the canals was pooled. After sampling, the sows were treated medically. Milk samples were collected from at least 4 different glands of each sow. A follow-up clinical examination was done 2 days later and milk sampling was repeated 2, 7 and 21 days later. Sows not showing symptoms of disease were milk-sampled as healthy controls but in these cases no clinical examination was carried out. Healthy sows were sampled during working hours while diseased sows were sampled regardless of time of farrowing. The samples collected during working hours were immediately analysed for total and differential

cell counts and pH-value. Milk samples collected outside working hours were stored cold or at -20°C until analysed.

All sows included in paper IV were clinically examined on the day of weaning and one week after weaning, with the exception of the sows that were immediately sent for slaughter after the first examination. On the day of weaning, the rectal temperature was measured before further examinations were performed. Body condition as well as the hoof condition were scored and open wounds in the scapular region were registered. Milk production from the mammary glands, full, partial or ceased, was estimated based on the distension of the individual gland. The mammary glands and teats were carefully examined and special attention was focused on injuries of the apex of the teat, skin lesions of the mammary glands and abnormal palpatory findings of the glands. The teat injuries were scored as slight, moderate or severe. Teat injuries characterized as slight were small abrasions at the apex of the teat. A moderate wound was extended over a larger area of the apex of the teat, while severe wounds were characterized by loss of tissue from the apex of the teat. Severe teat injuries could also include eczematous inflammation of larger parts of the skin of the teats. Palpatory findings indicating mastitis were described in detail and also scored as slight, moderate or severe.

Milk samples were collected from mammary glands with teat injuries and (or) from glands with palpatory findings. Samples from clinically normal mammary glands were collected as controls. The milk samples were collected with an identical procedure as described in detail in papers I, II and III. The milk samples were kept on ice until arrival at the laboratory.

The 11 sows included in paper V were clinically examined at the day of weaning in a way identical to that described in paper IV. The whole udder was examined and special attention was focused on the mammary glands with palpatory findings indicating chronic (granulomatous) mastitis. Abscesses and ulcera in mammary glands were scored as severe palpatory findings.

Milk samples were collected from the sows as described in paper IV, with the exception that milk was rarely possible to obtain from glands with abscesses and granulomas.

Bacteriological examination

In papers II and III, approximately 10 µl of the colostrum and milk was spread onto blood agar containing 5% horse blood and onto agar containing 1% lactose. The agar plates were examined after 24 h at 37°C. Duplicate blood agar plates were incubated anaerobically for 24 h at 37°C. Bacterial colonies were identified macroscopically and microscopically. Coliforms were identified by API 20E biochemical profiles (API 20E S.A., La Balmes Grottes, France) (Paper III). Identified coliform bacteria were also analysed serologically regarding O-antigen and fimbrial structures. When the agar plates were examined, >100 CFU/inoculum (colony forming units), revealed a profuse growth of *E. coli*, 10-100 CFU/inoculum gave moderate growth of *E. coli* and <10 CFU/inoculum sparse growth of *E. coli*. Beta-haemolytic streptococci and staphylococci were identified by use of the Lancefield system and DNAse test, respectively.

In paper IV, the milk samples arrived at the laboratory within 3 h (Mastitis Laboratory, National Veterinary Institute, Uppsala, Sweden). The milk samples were cultured and examined basically according to Nordic recommendations on examination of bovine quarter milk samples (Klastrup & Schmidt Madsen, 1974).

Milk samples collected in paper V were spread on blood agar plates for aerobic and anaerobic examination within 1 hour after collection. These procedures were performed on the farms. 10 µl of the collected milk was streaked onto bovine blood agar plates (5%, v/v, containing 0.5 g of aesculin per 1 litre of agar). The milk samples were also examined anaerobically after cultivation on Fastidious Anaerobic Agar (F.A.A.) in BBL Gas Pak+^{TM} Anaerobic system with Envelopes containing Palladium Katalyst (Becton Dickinson, Cockeysville, U.S.A). The agar plates were delivered to the laboratory (Department of Veterinary Microbiology, Section of Clinical Microbiology, SLU, Uppsala) within 3 h and incubated immediately at 37°C. The aerobic cultures were examined after 24 h and 48 h. The anaerobic cultures were examined after 48 and 168 hours and colonies of suspected anaerobic bacteria were subcultured under aerobic and anaerobic conditions for another 48 h. Thereafter, the anaerobic isolates were tested biochemically, using the Rapid ID 32A test-system, Bio Mérieux SA Marcy - l'Etoile (Lyon, France) or analysed using gas-chromatography and biochemical tests.

The eleven sows (Paper V) were slaughtered at the unit for sanitary slaughter (Scan-Farmek, Uppsala, Sweden) from 0 to 9 days after weaning. The udder was thoroughly washed before being removed from the carcass. The udder was then placed on ice in a plastic box and transported to the laboratory (Department of Obstetrics and Gynaecology). Tissue specimens from mammary glands earlier designated as clinically healthy and from glands with granulomatous lesions were collected after incision. The specimens were kept in petri dishes under aseptic conditions until preparations for bacteriological examination were performed. The surface of the mammary tissue specimens were heat-sterilized and material was collected from the centre of the tissue. Thereafter, the bacteriological examinations were the same as those described for the milk samples (Paper V).

From one herd, 3 sows severely affected by chronic mastitis were slaughtered at the unit for sanitary slaughter (Scan-Farmek, Uppsala, Sweden) between days 1 and 3 after weaning. The removal of the udder was carried out in the same way as described previously, and collection of material for aerobic and anaerobic bacteriological examinations initially followed the same procedures as described in paper V. In addition to these initial procedures, material was collected from the same tissue specimens in attempts to isolate granules from the pus or exudate. The flake- or granule-like material was placed in plastic tubes after collection and carefully washed in distilled water. This procedure was repeated 5 times. The remaining granules were then crushed and from this material, aerobic and anaerobic bacteriological examinations were performed as described previously. The anaerobic strains were further characterized at the Department of Bacteriology, National Veterinary Institute, Uppsala, Sweden. The final characterization of the phenotype profile was performed at the Department of Clinical Bacteriology, Sahlgrenska University Hospital, Gothenburg, Sweden. The strain (*Actinomyces-*

EF Group 78, CCUG 37626) was not previously isolated and was included in the culture collection as a 'new' strain of the genus *Actinomyces*. Further characterizations of the strain are in progress (FAME, protein profiles and sequencing of 16S rRNA in attempts to determine the phylogenetic relationships).

Cytology

In papers II, III, IV and V the total cell count was performed according to a method originally described by Prescott & Breed (1910) and modified based on the norms issued by the Sub-committee on Screening Tests, United States National Mastitis Council (1968).

Two smears were prepared from each sample on carefully cleaned slides. 0.01 ml of milk was placed on the slide by means of a microsyringe. Each drop was spread over an area of 0.5x2 cm (1 cm^2) and allowed to dry overnight. Methanol was then used as fixative and the smears were again allowed to dry until the next day. On day 3, the fixed smears were immersed in Newman's stain and allowed to dry overnight. On day 4, the stained smears were carefully washed in water and again allowed to dry until day 5 when total cell counts (Papers II, III, IV and V) and differential counts of polymorphonuclear leucocytes (PMNLs) (Papers II and III) were carried out at a magnification of x1000 under oil in a light microscope.

In papers IV and V, acridine orange (AO) staining of unwashed mammary secretion and fluorescence microscopy for differential cell counting (PMNLs) was performed according to the method described by Magnusson et al. (1991). Briefly, 20 µl of mammary secretion was diluted with 100 µl AO. Two hundred cells in the suspension were counted in a microscope equipped with UV light and fluorescence outfits (Dialux 20, Leitz Wetzlar, Germany).

The lowest cell class limit was established from bacteriologically negative samples by calculating the mean ± 2 SD, and was found to be close to $10x10^6$ cells/ml (Paper II). This cytological threshold level was further utilized in paper III in the definition of mastitis, i.e. mammary glands were considered to be mastitic if TCC exceeded $10x10^6$ cells/ml.

pH

The pH of fresh colostrum and milk, delivered immediately to the laboratory, was determined with a digital pH-meter (Orion Research model 701 A) (Papers II and III).

Histopathological examination

Tissue specimens (approximately 1 cm^3) were fixed in 4% neutral buffered formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin-eosin (HE) (Paper V). This procedure was also performed regarding tissue specimens from the mammary glands of the extra 3 sows included in the thesis.

Further examinations were carried out according to procedures used at the Department of Pathology (SLU). After histopathological confirmation, glands

clinically diagnosed with chronic granulomatous mastitis were denoted chronic pyogranulomatous mastitis.

Statistical analyses

Data handling, and the statistical analyses were performed by using the SAS software (SAS Institute Inc. 1989). Continuous traits were analysed by using analysis of variance (GLM procedures), and frequencies were analysed by using the chi-square test (Paper I) or Fisher's exact tests (Paper IV).

The statistical model used in paper I, included the effects of feeding level, health status and the interaction between feeding level and health status.

In papers II and III, logarithmic transformation (log_{10}) of the cytological variables was used with the aim of obtaining a more normal distribution. The statistical models in papers II and III included in different combinations the effects of feeding level, health status, day of sampling, lactation number and interactions between these effects.

In papers IV and V, the statistical models included the effects of herd, lactation number, health status and interactions. When repeated observations were present for each sow, the effect of sow was included in the models and was regarded as random. This means that the variation between sows was taken into account in the tests of significance of the fixed effects.

In the presentation of the results, the following levels of significance were used: N.S.= $p>0.05$, *= $p\leq0.05$, **= $p\leq0.01$, ***= $p\leq0.001$.

Results

Udder and general health conditions at farrowing and during the early lactation period

The effect of different feed allowances during late pregnancy on health status and performance of sows with special reference to APP (Paper I)

The incidence of APP was significantly more common in the control group of sows, where APP was diagnosed in 52 out of 195 lactations (26.6%) compared with 26 out of 181 (14.4%) lactations in the experimental group. Twenty-four of the sows in the experimental group and 11 sows in the control group were never diseased during the time of the experiment. The number of sows diseased at 1, 2 or 3 or more lactations in the control group and experimental group were 13, 9 and 6 versus 9, 3 and 3 animals, respectively.

The mean value of the rectal temperature was significantly higher in affected sows than in healthy sows from 1 day before farrowing until 1 day after farrowing, in both groups. A negative impact on the general condition and on the feed and water consumption was more pronounced among sows in the control group whereas a majority of the sows in the experimental group were registered as unaffected at the first clinical examination. This difference in clinical health status between sows in the two groups was obliterated at the second clinical examination. Udder changes were recorded in 88% of lactations in the experimental group and in 73% in the control group at the first examination and in 58% and 31%, respectively, at the second examination.

The length of the gestation period and the interval from weaning to first oestrus did not differ between healthy and agalactic sows. No significant differences in litter size at birth or at weaning were found between healthy and agalactic sows, although non-agalactic sows weaned a numerically higher number, 0.5 pigs/litter. The agalactic sows farrowed a significantly higher number of stillborn piglets compared with the non-agalactic sows, 1.0 versus 0.6 piglets/litter. Twenty-six of 39 sows in the experimental group and 32 of 39 sows in the control group remained in the trial throughout the designated 6 lactations.

TCC, PMNLs and pH in bacteriologically negative colostrum and milk obtained from sows clinically healthy with or without individual mammary glands subclinically infected with E. coli *or from sows suffering from APP (Papers II and III)*

The majority of the TCC values, 87.3%, obtained from bacteriologically negative colostrum and milk samples (Paper II), were below $10x10^6$ cells/ml, the designated cell class limit above which mastitis was defined. Another two cell class limits were also used in this study, $10\text{-}19.99x10^6$ cells/ml and $>19.99x10^6$ cells/ml. TCC values within the two latter cell classes in 63.4% and 58.8%, respectively, were preceded by cell counts below the threshold of $10x10^6$ cells/ml and in 46.3% and 58.8% succeeded by cell counts below the threshold. In another 26.8% and 17.6%,

neither the preceding nor the succeeding cell counts exceeded the threshold. A majority of the TCC values above $10x10^6$ cells/ml were obtained from a limited number of sows.

The mean of the TCC values below the threshold of $10x10^6$ cells/ml increased significantly from day 1 to day 3 ($1.23x10^6$ versus $1.86x10^6$) followed by a significant decrease to day 22. When cell counts from all cell classes were included, the TCC also increased significantly from day 1 to day 3. The TCC on day 22 was still on a significantly elevated level compared with that on day 1 ($2.10 x10^6$ versus $1.38 x10^6$).

In the cell class below $10x10^6$ cells/ml, the percentage of PMNLs decreased significantly from 58.5% on day 1 to 49.0% on day 3 of lactation. Thereafter, the percentage of PMNLs further decreased to 42.2% on day 8 ($p<0.05$) followed by a significant increase to 50.0% on day 22. When all cell classes were included, the PMNLs decreased significantly from 59.6% on day 1 to 49.1% on day 3, thereafter no further significant changes occurred.

The pH in colostrum and milk with a total cell content below the threshold value, increased significantly from 6.17 to 6.55 between days 1 and 3 and further to 7.01 on day 8, followed by a significant decline from day 8 to day 22 (7.01 versus 6.86). When pH-values from all cell classes were included, the pH-values remained stable and the changes between sampling days were almost at the same level.

In bacteriologically negative colostrum and milk samples collected from APP sows and from healthy sows but subclinically infected with *E coli*, the TCC-values increased significantly from day 1 to day 3 and thereafter decreased to day 8 (Paper III). The TCC-values from colostrum and milk samples on day 1 were significantly higher in APP sows ($2.27x10^6$) than in healthy sows with or without subclinical *E. coli* infected mammary glands ($1.51x10^6$ and $1.38x10^6$, respectively). On day 3, the TCC was significantly higher in APP sows ($4.86x10^6$) than in healthy sows without subclinical infections ($3.18x10^6$).

On day 1, the percentage of PMNLs in bacteriologically negative colostrum from clinically healthy sows without subclinical *E. coli* infections was significantly higher than in colostrum from subclinically *E. coli* infected sows or from APP sows (59.6% versus 43.5% and 48.3%, respectively). The percentage of PMNLs within the 3 groups thereafter decreased to values between 36.5 and 50.0.

The pH-values on days 1 and 3 were significantly higher in samples from APP sows than in the other 2 groups of sows (6.29 versus 6.18 and 6.18, respectively). There were significant increases in pH between days 1 and 3 and between days 3 and 8 in all 3 groups of sows.

The lactation number had a significant influence on TCC below $10x10^6$ cells/ml (Papers II and III) and on PMNLs (Paper III), an influence that also tended to be significant on the pH (Paper III). The APP sows showed a significantly higher mean pH than the other 2 groups of sows (Paper III). The TCC increased significantly from the first lactation ($1.01x10^6$), reaching a maximum in lactations 4 and 5 ($1.85x10^6$ and $1.78x10^6$, respectively) (paper II). Within the cell class below $10x10^6$ cells/ml, the PMNL percentage increased significantly from the first

lactation (42.8%) to lactations 3 to 6 (paper II). In lactations 2 to 5, the PMNLs fluctuated between 49.3% and 55.1%.

Neither of the two feeding regimes influenced the TCC, the PMNLs or the pH on the consecutive 4 sampling occasions (Papers II and III), except for higher TCC-values in APP sows in the control group than in APP sows in the experimental group (Paper III).

TCC, PMNLs and pH in colostrum and milk obtained from sows suffering from APP or clinically healthy but subclinically infected with E. coli *(Paper III)*

In sows suffering from APP, *E. coli* was diagnosed in 41 out of 51 (80%) lactations in the control sows and in 22 out of 26 (84%) in the experimental sows. In a few cases other bacterial infections were diagnosed, such as α- and β-haemolytic streptococci, *Staphylococcus* spp., anaerobic grampositive cocci, and *Enterobacter agglomerans*.

Among clinically healthy sows, *E. coli* infection was diagnosed in samples from mammary glands on the first sampling occasion in 11 out of 37 (30%) lactations in the control group and in 19 out of 59 (32%) lactations in the experimental group.

In sows suffering from APP and in sows that were healthy but subclinically infected with *E. coli*, growth of *E. coli* was revealed only on days 1 and 3 of lactation. In sows suffering from APP, the intensity in growth of *E. coli* was 63% profuse, 18% moderate and 19% sparse on the first sampling day. On day 3 of lactation, *E. coli* was isolated from 35% of the milk samples. Six % showed profuse growth, 13% moderate and 16% sparse. In sows that were clinically healthy but subclinically infected, the intensity of *E. coli* growth was 52% profuse, 34% moderate and 14% sparse on the first sampling day. On day 3 there was moderate growth of *E. coli* in 21% and sparse growth in 17% of the mammary glands.

In sows that were clinically suffering from APP, 87 mammary glands out of 116 infected with *E. coli*, and in the subclinical group, 17 out of 29 infected with *E. coli*, exceeded the TCC threshold of $10x10^6$ cells/ml already on the first sampling day. In sows clinically suffering from APP and infected with *E. coli*, the average TCC was $82.27x10^6$ on day one. The TCC decreased gradually from day 1 to day 22, the decrease being significant between days 3 and 8. In sows subclinically infected with *E. coli*, the average TCC in colostrum samples was $156.65x10^6$, followed by a significant and gradual decrease to $3.28x10^6$ on day 22. A comparison between the 2 groups of sows on day 1 of lactation revealed a numerically higher ($p=0.08$) TCC-value in the subclinical ($156.65x10^6$) than in the clinical ($82.27x10^6$) sows. On subsequent sampling days, the TCC values were significantly higher in the clinically infected sows than in the subclinical sows.

The percentage of PMNLs on day 1 was numerically higher ($p=0.0581$) in the subclinically infected sows than in the clinically infected sows (79.0% versus 65.8%). Within both clinical groups, there was a decrease from day 1 to day 3, the decrease being significant in the subclinical group, and thereafter, stabilizing on a level between 54.0% and 43.4%.

The pH-values in colostrum and milk samples collected from sows clinically (APP) or subclinically infected with *E. coli*, gradually increased between days 1 and 8, the increase being significant between day 3 and day 8, thereafter stabilizing on the day 8 level.

The 2 different feeding regimes did not influence the TCC, the percentage of PMNLs, or the pH in sows clinically (APP) or subclinically infected with *E. coli*.

The lactation number did not influence the TCC in the 2 clinical groups of sows, whereas it did have a significant influence on the PMNLs. During the 6 consecutive lactations, the sows with clinically or subclinically infected *E. coli* mammary glands showed a PMNL varying from 38.7 to 80.0%. Within both groups of sows, the percentage of PMNLs at the sixth lactation was significantly lower than in the preceding 5 lactations.

It was further possible to compare results from bacteriological and cytological examinations from 26 mammary glands emanating from 11 sows during two or more consecutive lactations. In the first lactation observed, and on the first sampling occasion, the bacteriological examination of all these 26 glands revealed *E. coli*, in sparse, moderate or profuse growth. No growth of *E. coli* was obtained when sampling was repeated for the third time (day 8). During the third and fourth samplings, 10 mammary glands had ceased in producing milk, and the TCC had decreased in 8 of the remaining 16 glands. When milk sampling was repeated from the same 26 mammary glands on the subsequent lactations, *E. coli* was isolated from 8 mammary glands, of which 6 revealed a TCC exceeding $10x10^6$ cells/ml. From the same sow, milk was sampled from the same mammary gland in two different lactations, and the isolated *E. coli* strains belonged to different serotypes (Pedersen Mörner et al., 1997).

Relationship between clinical mastitis and results from bacteriological and cytological examinations

When the diseased sows in the experimental and control feeding groups were clinically examined, swelling, reddening and (or) soreness were found to be common symptoms.

In APP sows and irrespective of feeding regimes, infections with major pathogens such as *E. coli* and *Staphylococci* spp., revealed clinical symptoms of swelling, reddening and (or) soreness in 51% of the mammary glands at the first clinical examination (Tables 2 and 3). Mastitis (TCC>$10x10^6$ cells/ml) was revealed in approximately 45% of the mammary glands in the 2 groups of sows.

When laboratory diagnosis, *E. coli* and a TCC>$10x10^6$ were compared with the clinical diagnosis of mastitis on the first sampling occasion in APP sows in both groups it was demonstrated that 38 of 87 (44%) of the mammary glands showed swelling, reddening and (or) soreness (Paper III, pp. 300-301).

Profuse growth of *E. coli* was often associated with a rise in TCC exceeding $10x10^6$ cells/ml.

Table 2. Experimental group of sows at first clinical examination post partum. Clinical status of 54 mammary glands recorded as swelling/reddening/soreness (s/r/s) and compared with bacterial result and cell count classes (TCC <10, TCC>10). The results emanate from 23 of 26 diseased lactations with at least one mammary gland clinically recorded as s/r/s

Bacterial result				Cell count (10^6)	
No growth	Mixed flora	*E. coli*	Other growth	TCC<10	TCC>10
20				16	4
	6			4	2
		25		7	18
			2	1	1
				1[a]	
				29	25

a/ Data incomplete - no bacteriological examination.

Table 3. Control group of sows at first clinical examination post partum. Clinical status of 63 mammary glands recorded as s/r/s and compared with bacterial result and cell count classes (TCC <10, TCC>10). The results emanate from 36 of 52 lactations with at least one mammary gland clinically recorded as s/r/s

Bacterial result				Cell count (10^6)	
No growth	Mixed flora	*E. coli*	Other growth	TCC<10	TCC>10
20				18	2
	10			9	1
		27+2[b]		7	20
			2	1	1
					2[a]
				35	26

a/ Data incomplete - no bacteriological examination. b/ Data incomplete - no cell counting.

Table 4. Clinical estimation of milk production by assessment of the degree of distension of mammary glands. The milk production was scored as full, partial or ceased. Twenty-five sows that contributed with 2 subsequent lactations during spring and autumn were compared

Spring			Autumn		
Full	Partial	Ceased	Full	Partial	Ceased
187			149	11	27
	55		33	10	12
		105	47	14	44
187	55	105	229	35	83
(53.9%)	(15.9%)	(30.2%)	(66.0%)	(10.1%)	(23.9%)

Udder health at the time of weaning

Frequency of teat injuries and abnormal mammary gland consistency at weaning (Paper IV)

Clinical estimation of milk production showed that more than 80% of the thoracic pairs and first abdominal pair were in full production at the end of the lactation. A comparison between the 25 sows that contributed with 2 consecutive lactations showed that the milk production status from individual mammary glands varied between spring and autumn (Table 4).

The percentage of lactations with teat injuries and (or) udder skin lesions was 60%, irrespective of lactation number, while the percentage of lactations with palpable changes increased significantly with increasing lactation number, reaching 61% at lactation numbers ≥3. Twenty-two of 99 lactations did not exhibit either teat injury or palpable mammary changes.

On the day of weaning, 21 out of 162 mammary glands with teat injuries were scored as severe, while 27 out of 85 mammary glands were scored with severe palpable changes. The remaining teat injuries and palpable changes were classified as moderate or slight. The teat or udder skin injuries varied in frequency from 16.3% in the 1st pair of teats to 10.9% in the 6th pair. The 162 observed injuries corresponded to 11.6% of all mammary glands examined. Palpatory changes of the mammary gland tissue were most frequently found in the first pair of mammary glands (9.4%) and varied from 2.5% to 9.4% within the seven pairs of mammary glands. Palpatory changes were found in 6.1% of the total number of glands examined.

On the day of weaning, 21 teat injuries and (or) udder skin injuries were scored as severe. Seven days post weaning, only one teat was still severely injured. Half the total number of mammary glands with palpable changes at weaning, were found to be clinically normal at 7 days post weaning, while 15 of the 27 mammary glands with severe palpable changes at the day of weaning also showed severe palpable changes 7 days later.

Twenty out of 47 (42%) milk samples collected from clinically normal mammary glands, and 25 out of 56 (45%) milk samples collected from mammary glands and teats with clinical changes, yielded growth of bacteria regarded as potential pathogens relevant for mammary gland infection. The bacterial flora was dominated by non-haemolytic as well as α- and β-haemolytic streptococci. The β-haemolytic streptococci were isolated more frequently from mammary glands and teats with clinical changes. When the sows were re-examined 7 days post weaning, secretions could be squeezed out from 10 glands of 10 sows. At weaning all these 10 glands had been clinically described with udder skin lesions, teat apex injuries or atrophy of the gland. Coagulase positive and coagulase negative staphylococci, β-haemolytic streptococci, *Actinomyces pyogenes,* and *Fusobacterium necrophorum* were isolated 7 days post weaning from these glands.

The TCC in milk from clinically normal mammary glands was almost significantly lower than TCC from glands and teats with clinical changes. The numbers of PMNLs and the percentage of PMNLs were significantly or almost significantly higher in milk from the abnormal glands and teats. The TCC in milk from clinically normal glands without bacterial growth was significantly lower than the TCC in milk without bacterial growth but emanating from clinically abnormal mammary glands and teats. When the same milk samples were compared, the PMNLs expressed as absolute or percentage values showed a significant or an almost significant difference.

Chronic granulomatous mastitis (CGM) in sows with reference to clinical findings, bacteriology, cytology and histopathology (Paper V)

Clinical estimation of milk production revealed full production in 86.4% of the two thoracic pairs of mammary glands while production in the remaining pairs decreased caudally with the pair.

Thirteen mammary glands with ceased milk production were described as CGM and most of these glands were found in the abdominal pairs of mammary glands. Eight mammary glands with palpable changes, 2 of which were described as CGM, were found within the group with partial milk production. An average of 1.5 mammary glands per sow were described as CGM.

Mammary glands described as CGM were firm, moderately enlarged and distorted by a few to numerous irregularly dispersed firm nodules measuring from 1 to several centimetres in diameter. Some of the nodules were ulcerated. Microscopically, granulomatous formations consisted of solitary or multiple solid eosinophilic bodies with club-like protrusions surrounded by a broad zone of debris and neutrophils, and further demarcated by a reactive fibrous tissue.

Milk samples and tissue specimens were collected from 24 normal mammary glands. Bacteriological examination of milk samples revealed 8 samples with no growth, 12 with mixed flora, and 4 with growth of *Streptococcus* spp. Culturing of corresponding mammary tissue revealed 20 specimens with no growth and 4 with growth of mixed flora. Histopathologically, 23 specimens were normal while lesions of acute mastitis were evident in one gland.

Twenty-eight mammary glands with clinical changes were examined, of which 11 exhibited minor and 17 major clinical changes. Ten glands could not be sampled for milk, 8 of which had major clinical changes. Bacteriological examination of tissue specimens of 2 of these 10 mammary glands were negative and from the remaining 8, bacteria such as *A. pyogenes*, *F. necrophorum* and *Staphylococcus* spp. were isolated. Seven milk samples revealed no bacteriological growth while bacterial examination of tissue specimens from corresponding glands yielded isolates of *A. pyogenes*, *F. necrophorum* and *Prevotella* spp. from two glands, *Peptostreptococcus* from one gland, and negative growth from the remaining 4 mammary glands. A mixed bacterial flora was found in 7 milk samples, while culturing from the corresponding mammary tissue was negative in 5 cases and from the remaining 2 mammary glands *Staphylococcus* spp. and *A. pyogenes* were isolated. Pathogens were recovered from 4 milk samples and the bacteria species were as follows: *Enterococcus* sp. in mixed flora with *F. necrophorum*, *Streptococcus* spp. and *S. aureus*. Only *S. aureus* was isolated from the tissue specimen. Taken together, bacteriological examination of tissue specimens showed no growth in 13 samples and growth of pathogens in 14 samples. Thirteen glands of 14 with growth of pathogens had earlier been examined and found to have major clinical changes. The histopathological analyses showed normal involution in tissue specimens from 9 mammary glands, chronic pyogranulomatous mastitis in 11 glands, 5 glands with other histopathological changes and 3 glands were not analysed.

The TCC in milk from clinically normal and abnormal mammary glands was not significantly different ($37.17x10^6$ vs $41.78x10^6$, respectively). No significant differences were found regarding the PMNLs. The TCC in milk from clinically normal mammary glands with no bacterial growth was numerically lower ($28.58x10^6$) compared with the TCC in milk with growth of pathogens ($47.43x10^6$), while the differences in PMNLs ($0.82x10^6$ vs $12.90x10^6$ and 0.59% vs 41.72%, respectively) were significant. The milk from clinically abnormal mammary glands and teats revealed no significant differences, regarding TCC, PMNLs or percentage of PMNLs, when the bacteriological results were compared.

Results from an additional study of CGM in 3 sows

This study comprised 3 sows which were clinically examined before slaughter and examined post mortem according to relevant bacteriological and histopathological procedures (Thesis, pp. 24-25). Direct microscopy of washed exudate from all 3 sows showed single or clusters of Gram-positive branching filamentous rods. Similar Gram-positive branching filaments were seen in smears from cultures of the bacteria. Culturing under aerobic conditions yielded growth of *S. aureus* from exudate of the first sow, growth of *S. aureus*, *P. multocida* and *Streptococcus suis* from the exudate and *S. aureus* and *P. multocida* from the washed flakes of the second sow, while culturing of material from the third sow showed small β-haemolytic colonies with a bacterial appearance described as Gram-positive pleomorphic rods. The washed material from all three sows was cultured under anaerobic conditions and revealed considerable differences in colonial morphology. The bacteria were dominated by Gram-positive pleomorphic rods. Phenotypical characterizations of the anaerobic bacteria isolated revealed a 'new' strain of the genus *Actinomyces* (*Actinomyces*-EF Group 78, CCUG 37626). A description of the phylogenetical relationships remains to be elucidated.

Histopathological examinations revealed chronic pyogranulomatous mastitis in tissue specimens from all 3 sows.

General discussion

The main objectives of the present investigations were to study (1) the udder of APP sows (agalactia post partum) or sows remaining clinically healthy during the early lactational period and (2) sows with no or escalating clinical signs of impaired udder health at the time of weaning. During the post partum period, the udder health status, the general health condition and the performance of sows were evaluated with special reference to the potential influence of different feed allowances during late pregnancy.

Monitoring of udder health comprised thorough clinical examination of individual mammary glands and bacteriological and cytological examinations of colostrum and milk. In addition, measurement of pH in milk during the early lactational period and histopathological examination of tissue specimens collected from mammary glands of slaughtered sows at the time of weaning were performed.

Early development and diagnosis of agalactia post partum (APP)

In the present study, the rectal temperature was significantly elevated already 1 day before parturition in sows which developed APP, irrespective of whether the sows were exposed to the control or the experimental feeding regimes (Paper I, Figures 2a and 2b). The number of piglets born alive did not differ between clinically healthy and agalactic sows. However, a difference in number of stillborn piglets per litter was found between agalactic and non-agalactic sows (1.0 vs 0.6, respectively) (Paper I, Table 6). This latter observation is in accordance with Hermansson et al. (1978a), Jorsal (1983) and Bäckström et al. (1984). The significant increase of the rectal temperature before farrowing and the significantly larger number of stillborn piglets in sows later developing APP, indicate that APP is established already before parturition. Wegmann (1985), isolated *E. coli* from mammary glands just prior to farrowing, which further strengthens the hypothesis of an early initiation of APP. Whether an early establishment of APP interferes directly with the vitality of the foetuses and (or) prolongs the labour, resulting in an increased number of stillborn piglets, cannot be confirmed from the present investigation.

For an early detection of APP it is crucial to establish a threshold above which the rectal temperature predicts the disease and only to a minor degree is influenced by different physiological and environmental factors. The advantage of monitoring the rectal temperature is firstly that it provides us with clinical data free of subjective assessments, and secondly that it is a sensitive indicator of inflammation. In clinically healthy lactating sows, Elmore et al. (1979) reported that a 95% confidence interval included body temperatures from 38.9°C to 39.5°C. In our study, several observations support that an acceptable accuracy in prediction of APP was obtained when the threshold of the rectal temperature was set at 39.5°C (Paper I). *E. coli* bacteria were isolated together with a TCC exceeding $10x10^6$ cells/ml in 64% of the APP lactations, which clearly demonstrates that *E. coli* mastitis is a major component implicated in the etiology

of APP. If we include both *E. coli* infected sows with a delayed TCC rise (day 3), and sows infected with other bacterial species (TCC>$10x10^6$), 79% of the APP sows (lactations) were found to suffer from mastitis. The corresponding figure from the sows with a rectal temperature ≤39.5°C was 30%.

The general condition of the APP sow was graded according to degree of severity of different symptoms. At the first clinical examination, 55% of the sows (lactations) were noted to have some degree of symptoms. Less than 40% of the sows (lactations) were observed to have decreases in feed- and water consumption (Paper I, Table 3). Normally, sows eat less feed at the time of farrowing and scoring the water consumption might give incorrect values. Moreover, sows suffering from APP were scored regarding their feed and water consumption, in addition to their general condition, in attempts to evaluate the impact of the two feeding regimes imposed upon them (Paper I, Table 3). Although, the general condition was subjectively graded it clearly indicated that sows fed according to the control feeding regime and developing APP were clinically more severely affected than sows fed according to the experimental feeding regime. More detailed information will be given later, when comparing the effects of the two different feeding regimes.

The assessment of udder health was performed by a thorough clinical examination of the udder. Signs of swelling, hardening and soreness that indicated mastitis were recorded. In APP sows and irrespective of feeding regimes, infections with major pathogens, predominated by *E. coli*, revealed clinical symptoms in 51% of the mammary glands (Thesis, p. 31, Tables 2 and 3). Similarly, mastitis expressed by an increased cell count (TCC>$10x10^6$ cells/ml) revealed clinical symptoms in approximately 45% of the glands. If the bacteriological and cytological analyses were combined, 38 (44%) out of 87 mammary glands infected with *E. coli* and a concomitant TCC exceeding $10x10^6$ revealed clinical symptoms of mastitis (Paper III, pp. 300-301). These comparisons clearly indicate a limited conformity between results obtained from clinical examination and results from bacteriological and cytological examinations. Although somewhat discouraging, it is the author's opinion that a clinical examination of the udder should always be carried out in attempts to set a preliminary diagnosis of mastitis. Only tentative interpretations are advisable before results from the bacteriological and cytological examinations have been compared and evaluated. Sandstedt & Carlquist (1952) suggested that an increased consistency in the mammary glands might be an expression of oedema, a statement that to some extent might account for the discrepancies revealed in the present study between clinical and laboratory diagnoses. The influence of different feeding intensity will be a subject for discussion later in this text.

Factors influencing the cell content in bacteriologically negative colostrum and milk

Knowledge of factors influencing levels and variations of TCC and PMNLs in bacteriologically negative samples of colostrum and milk from clinically healthy sows is of great importance when comparing and interpreting differences between

normal levels of the cell populations and levels indicating inflammation. In the present study, the mean values representing normal cell content levels in colostrum (day 1) and milk emanated without exception from bacteriologically negative samples collected from clinically normal mammary glands in clinically healthy sows. It is also of crucial importance to define an upper cell limit which, when exceeded, clearly indicates an inflammatory process. In the present study, this threshold, $10x10^6$ cells/ml, was established by adding approximately 2 standard deviations to an estimated mean of the TCC. Between 4% and 21% of the TCC-values, depending on the sampling day, exceeded the designated threshold of $10x10^6$ cells/ml. Colostrum and milk both derive from 2 separate duct systems within the mammary gland (Schummer et al., 1981; Elmore & Martin, 1986). Conditions of the separate systems of the mammary gland may vary and thus contribute to variations in cell counts as may irregular sucking. The increases in TCC, seen in our study was of transient character, since the subsequent TCC-values were below the threshold in more than 50%. Retrospectively, Wegmann (1985) observed that glands not being suckled increased their mean cell count in milk to levels twice the threshold level already 5 days post partum.

In the present study, the TCC increased significantly from day 1 to day 3 ($1.23x10^6$ cells/ml vs $1.86x10^6$ cells/ml) and decreased thereafter gradually to day 22 in clinically healthy sows. The level of TCC on day 1 was higher compared with observations presented by Hurley & Grieve (1988), within the same range as reported by Wegmann (1985) and by Drendel (1991) but lower than in other studies (Ross et al., 1981; Evans et al., 1982; Magnusson et al., 1991). An increase in TCC from day 1 to day 3 is in accordance with previous investigations (Wegmann, 1985; Schollenberger et al., 1986). The subsequent decrease in TCC is also in accordance with earlier reports (Wegmann, 1985; Schollenberger et al., 1986; Magnusson et al., 1991). Most sows successively increase their milk production during the lactation period and reach a peak in production between weeks 3 and 5 (Allen & Lasley, 1960). In the present study, an elevation of milk production might have contributed to a slight dilution effect of the TCC between days 3 and 8. Comparing cows, Brolund (1985) also underlined the significant influence of non-bacterial factors such as daily milk yield and stage of lactation as causes of variation of the somatic cell counts. Also in sows, other unknown factors such as different breeds, might influence the variations in cell content during the lactation period. These factors have to be further elucidated.

Observations from the present study showed that the PMNLs accounted for 60% of the cells in colostrum. The level of PMNLs in colostrum is well in accordance with results from some earlier studies (Lee et al., 1983; Schollenberger et al., 1986; Hurley & Grieve 1988; Magnusson et al., 1991). Weber &Ferguson (1982) reported considerably higher and Evans et al. (1982) slightly higher percentages of PMNLs in colostrum. The reason for their deviating results is unknown. Together with the macrophages the PMNLs are the predominant cells in colostrum from women (Ho et al., 1979) and from cows (Lee et al., 1980). The PMNLs act by their phagocytic properties in the first line of defence against micro-organisms. Recruitment of a sufficient number of effective phagocyting PMNLs might be more

important during the establishment of the lactation, taking into consideration that the sow is still under the influence of the physiological and endocrinological changes occurring at the time of parturition, compared with the remaining lactation period. In our study, the PMNLs in milk varied between 40% and 50% during the lactation period (day 3 to day 22). Concerning this period of the lactation, Schollenberger et al. (1986) described almost identical figures, while others registered a somewhat more pronounced decrease around day 20 of lactation (Evans et al., 1982; Lee et al., 1983). Wegmann (1985) reported an extremely low percentage of neutrophilic granulocytes in both colostrum and milk (<10%), possibly being a result of the staining and counting methods used by him differing to some extent from ours.

TCC and percentage of PMNLs were shown to increase with lactation number (Paper II, Table 4). There was a significant increase in TCC when lactation 1 ($1.01x10^6$ cells/ml) was compared with consecutive lactations 2, 4, 5 and 6. The TCC reached a maximum in lactations 4 and 5 ($1.85x10^6$ cells/ml and $1.78x10^6$ cells/ml), and thereafter decreased to the final lactation, number 6. The percentage of PMNL increased significantly from the first lactation, 43%, to successive lactations 3 to 6. In lactations 2 to 5 the PMNLs fluctuated from 49% to 55%. Drendel (1991) observed that the TCC and the number of neutrophilic granulocytes tended to be higher in multiparity sows when they were compared with first parity sows. These observations might indicate an increasing stimulus on the sow's immune system with elevated lactation numbers. This corresponds to observations in cows, where a systematic increase in base-level cell counts has been reported (Emanuelson & Persson, 1984; Brolund, 1985). Several factors were suggested to be involved, among them physiological age differences and effects of previously eliminated infections (Brolund, 1985). It should be underlined that our results emanate from consecutive lactations of the same sows. Nevertheless, as was underlined concerning the variations within lactations, more detailed information is desirable to elucidate and clarify the variations in cell content between consecutive lactations.

The two different feeding regimes during late gestation had no influence on the TCC or the percentage of PMNLs in colostrum and milk during the lactation (Paper II).

Hitherto, the results concerning the cell content in colostrum and milk have emanated from bacteriologically negative samples collected from clinically healthy sows. Also in APP sows and in clinically healthy sows, but with individual mammary glands subclinically infected with *E. coli*, it was possible to obtain colostrum and milk samples without bacterial growth (Paper III, Table 5). The TCC in bacteriologically negative colostrum was significantly higher in APP sows ($2.27x10^6$ cells/ml) than in clinically healthy sows or from clinically healthy but subclinically *E. coli* infected sows ($1.38x10^6$ cells/ml and $1.51x10^6$ cells/ml, respectively). In milk collected on day 3, a significant difference existed between APP sows and clinically healthy sows without subclinical *E. coli* mastitis. Finally, the differences in TCC between the 3 clinical groups were insignificant on day 22. The elevated TCC level in colostrum from APP sows was most probably caused by

a transient hypogalactic condition. This conclusion is further strengthened by the fact that there was a stepwise obliteration in the differences in TCC between the clinical groups of sows during the remaining sampling period up to day 22 when milk production was re-established. In contrast, the percentage of PMNLs in bacteriologically negative colostrum was significantly higher in clinically healthy sows (60%) than in APP sows or in subclinically *E. coli* infected sows (Paper III, Table 6). No significant differences in PMNLs were revealed during the remaining sampling period. The lower percentage values of PMNLs in bacteriologically negative colostrum from APP sows or from clinically healthy sows with subclinical *E. coli* mastitic glands might indicate a redistribution of the PMNLs to the *E. coli* infected glands or, as will be discussed in later passages in this text, be a consequence of endotoxins causing a neutrophilic leucopenia in the blood (Nachreiner et al., 1972). It must be emphasized that the total amount of PMNLs available in bacteriologically negative colostrum in APP sows exceeded the total number of PMNLs present in mammary glands belonging to the healthy sows.

The effect of different feed allowances during late gestation on the occurrence of APP

In the present study (Paper I), sows given a restricted feed allowance (experimental group) during late pregnancy had a significantly lower incidence of APP (14.4%) compared with the control group of sows fed a standard diet (26.6%). The comparatively high incidence rates of diagnosed APP in both groups of sows might be explained by the very careful clinical observations of the sows, starting already 2 days before expected farrowing, and allowing detection of also mild cases of APP. In APP sows, *E. coli* infection of the udder was diagnosed in 80% of the lactations in the control group and 85% in the experimental group. In clinically healthy sows, infections of *E. coli* were as frequent in the control group (30%) as in the experimental group (32%). The percentage of APP lactations with growth of *E. coli* and cell counts exceeding $10x10^6$/ml colostrum and milk, were equal in the experimental and control groups (62% and 65%, respectively). A similar evaluation of the clinically healthy sows revealed that the percentages of lactations with subclinical *E. coli* mastitis in the experimental and control groups were equal (24%) if we extend the period for elevation of the TCC until day 3. Otherwise, the sows fed according to the experimental regime revealed a lower percentage of lactations with *E. coli* mastitis (10%) (Paper III, Table 2). Although sows in the experimental group were offered a restricted feeding regime, possibly causing milder symptoms not leading to clinical examination, the observations indicate that the restricted feed allowance did not camouflage sows suffering from APP. On the contrary, the figures strengthen the hypothesis of a correlation between a restricted feeding regime before farrowing and a decreased frequency of APP compared with the feed allowance offered the control group of sows.

Sows exposed to the control feeding regime and developing APP, were to a greater extent clinically depressed with a decrease in feed and water consumption at the first examination, whereas a majority of the sows fed according to the restricted regime were registered as unaffected (Paper I, Table 3). Although the

general condition was scored subjectively, it is clear that sows fed according to the control regime and developing APP were clinically more severely affected and probably more predisposed to develop APP compared with sows fed a restricted feeding regime (experimental group).

Udder changes were registered in 88.4% of agalactic sows in the experimental group and in 73.1% of agalactic sows in the control group (Paper I, Table 3). These figures are in accordance with the work of Ringarp (1960), but are higher than in the work of Hermansson et al. (1978a), who reported signs of mastitis in 45-52% of agalactic sows. A combination of bacteriological and cytological analyses, revealed 87 *E. coli* mastitic glands, out of which 38 (44%) had been examined with clinical symptoms of mastitis. When, comparing the two different feeding groups, 18 of 26 (69%) glands in the experimental group of sows and 20 of 61 (33%) glands in the control groups of sows were clinically abnormal. These latter observations indicate an impact of feed allowances during late gestation, causing a more pronounced 'physiological' swelling of the mammary glands, which renders it more difficult to palpate.

An overall analysis revealed that the 2 feeding regimes did not influence upon the PMNLs or the pH in milk from glands with *E. coli* mastitis or in milk from bacteriologically negative glands. On the other hand, significantly higher TCC in bacteriologically negative milk in the control group of sows compared with the experimental group ($3.62x10^6$ cells/ml vs $2.69x10^6$ cells/ml, Paper III, Table 9) was noted.

The result from the present study confirmed the results from Sandstedt et al. (1979), Møller Jensen (1981) and Sandstedt & Sjögren (1982), who reported a decrease in the incidence of agalactia post partum among the sows offered only 1 kg feed per day during the final 3 weeks of the gestation period. In a recent study, Neil (1996) studied sows given a standard diet during the gestation period, but which at farrowing were either fed *ad libitum* or according to a conventional feeding standard with a stepwise increase in daily feed allowance. The incidence of agalactia was significantly higher in the sows fed *ad libitum* from the day of farrowing compared with the sows fed according to the standard model after farrowing. Sows fed a diet low in crude protein prior to farrowing were less frequently diseased in APP (Sandstedt, 1953; Sandstedt et al., 1979). In a later Swedish study, Göransson (1989a) reported that an increase in crude fibre content of sows' diet and offered to the sows during gestation, numerically reduced the occurrence of APP compared with sows receiving a diet with normal crude fibre levels. Göransson (1990b) also reported that an all-vegetable protein diet (defatted soy bean meal) compared with a standard diet containing fish-, meat- and bone-meal (both diets being offered to the sows during the last three weeks of gestation) almost significantly reduced the occurrence of APP (30% vs 50%). It might be pointed out that the incidences of APP were high in the two feeding groups of sows and reflected the criteria used when medical treatment was initiated against APP.

The results obtained in the present study indicate an impact of feeding intensity not only on the incidence but also on the severity of APP. A plausible hypothesis suggested by Gooneratne et al. (1982), is that a premature initiation of lactation

causes an engorgement of the mammary glands in connection with an impaired resistance to udder infections. According to the authors, the concentration of Na^+ was increased while the concentrations of K^+ and lactose were low in colostrum and milk collected from sows suffering from MMA. These changes in the concentrations of ions might be attributed to leakage through damaged cell membranes. In our study, Göransson (1990a) showed that sows fed according to the restricted feeding regime had significantly higher levels of fat but, on the other hand, also significantly lower levels of IgA in colostrum compared with sows fed according to the control regime. There was no evaluation or discussion of the possibility that the high fat level in colostrum from restricted-fed sows might be of protective value against potential pathogens such as *E. coli*. The higher fat levels were considered to reflect the metabolic status of these sows synthesising milk directly from body tissue. Mammary glands with impaired resistance to invading infections might be more vulnerable if we consider the potential hazards that exist when the udder is exposed to an environment contaminated by a faecal bacterial flora. Awad-Masalmeh et al. (1990) demonstrated identical O serotypes of *E. coli* in milk and faeces of one-fourth of 67 sows with coliform mastitis. A restricted feeding regime might partially exert its effect through a smaller amount of faeces produced and thus reduce the exposure of the teats to a contaminated environment (Bertschinger & Pohlenz, 1992). Bertschinger et al. (1990) reported that preventive measures taken in attempts to protect the udder against faecal contamination substantially decreased the number of mammary glands infected with *E. coli*, although a few mammary glands were still subclinically infected with *E. coli*. This *E. coli* mastitis did not cause any clinical signs of systemic effects. This observation indicates that some sows, despite the preventive measures taken, are predisposed to the *E. coli* infection so strongly incriminated in the etiology of APP, being a conclusion that might raise new hypotheses when studying the etiology of the APP.

Escherichia coli mastitis - clinical, bacteriological and cytological aspects

E. coli has been suggested to be the most common organism invading the mammary glands in sows directly pre- or postpartum. This invasion often leads to mastitis which, in the majority of cases, is implicated in the APP syndrome (Martin et al., 1967; Thurman & Simon, 1970; Armstrong et al., 1968; Bertschinger et al., 1977; Middleton-Williams et al., 1977; Ross et al., 1981; Wegmann, 1985).

Bacteriological examination of colostrum and milk from APP sows or from clinically healthy sows, yielded growth of *E. coli* from one or several mammary glands (approx. 80% and 30% of the lactations, respectively; Paper III). Healthy sows with *E. coli* were designated as being subclinically infected. Whether these *E. coli* bacteria were established before farrowing, as reported by Wegmann (1985), was not investigated in the present study. On the first sampling day, colostrum from mammary glands from clinically healthy sows as well as from APP sows yielded a profuse growth of *E. coli* in more than 50% of the samples. On day 3 of lactation *E. coli* was isolated from 35-40% of the mammary glands which previously had

yielded growth of *E. coli*, with a concomitant decline in the *E. coli* growth. The final elimination of the *E. coli* occurred between days 3 and 8. These results are well in accordance with Wegmann (1985), who presented results from a study where 8 multiparous sows were sequentially sampled for milk during a lactation period of 29 days. Subclinical mastitis was cytologically diagnosed in 41 mammary glands, of which 18 revealed growth of *E. coli*. The author observed that the *E. coli* was most frequently isolated from the mammary glands during the first and second days post partum. The spontaneous elimination took 0.5 to 4.5 days, with an average time of 2.2 days. My own results (Thesis, p. 30) also support the hypothesis that invaded mammary glands eliminate the *E. coli* bacteria. No infection and no inflammation persists from one lactation to subsequent ones. An interpretation based on these observations is that the *E. coli* mastitis in sows is of an acute character and imposed to a strict time-limit during the early lactation period. The bacteriostatic and bactericidal properties in colostrum and milk from unsuckled or mastitic glands was demonstrated to inhibit growth of both entero-pathogenic and udderpathogenic *E. coli* in vitro (Wegmann, 1985). On the contrary, when *E. coli* was cultured in colostrum and milk from clinically healthy suckled glands, no inhibition in growth was observed. The degree to which the mammary tissue might be destroyed and, consequently, the milk production decreased, was not estimated in our study. Nevertheless, a significant difference in within-litter standard deviation for weights at 3 and 6 weeks of age between clinically healthy and APP sows might indicate a negative influence of APP on milk production (Göransson, 1989b). Also Bertschinger et al. (1990) reported a transient decrease in daily weight gain for the first 4 days of life of piglets sucking mammary glands with subclinical mastitis compared with piglets sucking healthy mammary glands.

Serological analysis of O-antigens was performed in 69 randomly sampled isolates from the present investigation (Pedersen Mörner et al., 1997). Eighteen O-antigens were identified, while 35 of the 69 strains were serologically non-typable. Isolates of *E. coli* from the same sow and teat during the same lactation belonged to the same serotype, while isolates obtained from different teats in the same sow differed serologically. Armstrong et al. (1968) and Bertschinger et al. (1977) also reported that serotyping of *E. coli* strains isolated from mastitic milk of sows revealed a high heterogeneity. In the present study, observations of the same mammary glands being sampled for milk during subsequent lactations (Thesis, p. 30) revealed that the *E. coli* was repeatedly eliminated between days 3 and 8 in the lactations examined. Further, Pedersen Mörner et al. (1997) showed that strains of *E. coli* isolated from the same teat of a sow on two different lactations belonged to different serotypes. This clearly indicates that the ubiquitous *E. coli* might act as a potential pathogen. Bertschinger et al. (1990) convincingly confirmed that protection of the mammary glands against faecal contamination from the environment substantially reduced the incidence of puerperal mastitis mainly caused by *E. coli*.

The *E. coli* in mammary glands of APP sows caused high TCC in milk (Paper III). The clinically healthy sows affected by a subclinical *E. coli* mastitis showed

an even higher TCC on day 1 of lactation than the APP sows ($156.65x10^6$ cells/ml and $82.27x10^6$ cells/ml, respectively). Wegmann (1985) defined mammary glands as being mastitic if the TCC exceeded $5.0x10^6$ cells/ml and the percentage of neutrophilic granulocytes was ≥70. In his study, 18 of 41 mastitic glands were infected with *E. coli*. In the 41 mastitic glands, the TCC increased above $10x10^6$ cells/ml milk during the first days post partum. Since the sows remained clinically healthy in his study, the mastitis reaction should be classified as subclinical. In our study, APP sows with *E. coli* mastitis as well as sows with subclinical *E. coli* mastitis showed elevations of PMNLs in colostrum. The percentage level of PMNLs tended to be significantly higher in subclinical *E. coli* mastitis than in clinical *E. coli* mastitis. This might indicate a more successful migration of the PMNLs to the mammary glands in clinically healthy animals. Data reported by Löfstedt et al. (1983) indicated that susceptibility to *E. coli* mastitis was associated with deficiencies in number and function of the PMNLs in sows. In cows, a slow migration of neutrophils appears to be associated with the most severe cases of *E. coli* mastitis (Hill et al., 1979). The lower percentage of PMNLs in milk from mammary glands in the group of sows with APP might also reflect the decrease in segmented neutrophils observed in blood after intravenous or intramammary infusions of endotoxin (Nachreiner et al., 1972; Nachreiner & Ginther, 1974). Endotoxinemia was more prevalent in dysgalactic than in healthy sows (Morkoc et al., 1983). In mastitic mammary glands, Wegmann (1985) obtained levels of neutrophilic granulocytes exceeding 80% of the TCC on day 1 of lactation. After 2 days the percentage of neutrophilic granulocytes fell to levels below 70%, which is in accordance with results obtained in our study.

The lactation number had an overall significant influence on the PMNLs but not on the TCC in APP sows with clinical *E. coli* mastitis and in clinically healthy sows with subclinical *E. coli* mastitis. In both groups of sows, the sixth lactation revealed a significantly lower percentage of PMNLs. It can be emphasized that these findings deviate from results presented earlier, where the lactation number had an influence upon increases in TCC and PMNLs in bacteriologically negative milk obtained from clinically healthy sows (Paper II).

Mastitis at the time of weaning - predisposed by teat- and udder skin injuries?

During lactation, some authors claim that the most probable route for bacteria to enter the mammary tissue is through penetrating wounds of the skin of the mammary glands and teats, mainly caused by the sharp teeth of the piglets (Magnusson, 1928; Englert, 1961; Renk, 1962; Jones, 1980; Meermeier, 1987). The validity of the latter hypothesis ought to be further tested, since potential pathogenic bacteria might also be able to enter the mammary glands in sows by the galactogenic route.

The sucking behaviour of growing piglets is often a vigorous process starting already when they set up a 'teat order' (Fraser & Thompson, 1991). This competition continues throughout lactation (Fraser, 1990) and might be one of several factors that damage the mammary glands and teats and thus predispose to a

successively impaired health status of the udder of the sow. Teat damage might also occur in sows as a result of other external mechanical trauma (Done, 1980). Such external trauma might be caused by the perforated floor in the farrowing pen (Svendsen et al., 1984; Edwards & Lightfoot, 1986). Since perforated floors were uncommon in farrowing pens in the present study (Paper IV), the impact of the piglets' sucking behaviour seems to be more prominent. The ultimate consequence of severely impaired udder health in sows at the time of weaning or during the immediate post-weaning period might be the pathological transformation of glands into a condition denoted chronic granulomatous mastitis, CGM, (Paper V).

Observations from the present study indicate that the intensive nursing of the high milk-yielding pairs of mammary glands tended to be correlated with the highest frequency of teat injuries and udder skin injuries (Paper IV, Fig. 1, Table 4). The more severe teat wounds tended to be located in teat pairs 4-6. Whether the occurrence of more severe wounds in teat pairs 4-6 is related to more fighting among piglets managing to remain at the middle of the udder (Fraser, 1975), or to the short distance between the abdominal pairs of mammary glands and the floor, could not be elucidated in our study. Edwards & Lightfoot (1986) reported that the highest frequencies of 'teat cuts' were demonstrated in pairs 5-7, while almost no injuries were found on the three cranial pairs of teats. These 'teat cuts' were caused by the back hoofs of the sows when struggling to stand on the perforated floor.

A re-examination of mammary glands and teats 7 days after weaning revealed that 76% and 50% of the teat injuries and the palpatory changes of the mammary glands, respectively, had healed or disappeared. Surprisingly, even the severe teat injuries declined in severity during the first 7 days after weaning. Pierskalla et al. (1990) observed that 50-70% of all nodular alterations subsided during the first 5 weeks after weaning. Results from my study might to some extent add to the confusion, since it was revealed that another 57 glands (c. 4% of examined glands) were noted with palpatory changes 7 days after weaning (Paper IV, Table 5). These 57 glands were found to be in good order when clinically examined at weaning. In these three herds, the breeding units were provided with abundance of straw that made the environment comfortable for the sows and kept the udders clean, thus reducing the risk of the teat apex becoming infected by potential pathogens. The prevalence of palpable changes increased gradually from the first to the second and subsequent lactations (15%, 30% and 60%). The increases from the first to the second and from the first to the third and subsequent lactations were significant. These figures might indicate a successive negative impact on udder health under the precondition that a clinical examination truly reflects udder health. It must be underlined, as was done for clinical findings from the early lactational phases, that further confirmative bacteriological and cytological analyses are needed before conclusive interpretations are made. Further, it might be interposed that a dynamic variation in the degree of milk production from the same mammary glands was observed when two subsequent lactations were compared (Thesis, p.31, Table 4). Non-suckled teats cease to produce milk already 1-2 days after farrowing (Dellmeier & Friend, 1991). Drendel (1991) reported that milk collected from non-suckled mammary glands had increasing levels of cells already 5-6 days after

cessation of suckling. The remaining number of mammary glands producing milk is fairly well correlated to the number of piglets per litter. Therefore, at the time of weaning, interpretation of clinical findings of single mammary glands exhibiting a non-functional status should be done with care since they might indicate either a physiologically ceased status or cessation caused by pathological destructive processes resulting from severe bacterial infections. Taken together, interpretation of clinical observations implies that hesitancies might arise when trying to predict a long-term outcome of especially minor clinical findings and their significance. Repeated examinations of the mammary glands might to some extent provide better prediction of the long-term outcome of udder health and are therefore desirable. Large variations might exist between different herds, but conclusive knowledge is lacking.

Isolation and identification of the bacterial agents in teat wounds are of fundamental interest, since these bacteria might further enter into the mammary tissue. Bacteriological examination of milk collected from mammary glands with clinically abnormal consistency or from teats with various degrees of lesions were compared with the bacteriological outcome from clinically normal glands (Paper IV, Table 6). *Streptococcus* spp. and *Staphylococcus* spp. were isolated to the same extent, although β-haemolytic streptococci were more frequently isolated from abnormal mammary glands and teats. Milk secretions were squeezed out from a few glands with moderate to severe clinical findings 7 days post weaning (Paper IV, Table 6). Bacteriological examination of the secretion revealed *S. aureus*, *Streptococcus* spp. and the mammary gland pathogens *A. pyogenes* and *F. necrophorum* normally implicated in formation of abscesses in the mammary glands (Delgado & Jones, 1981; Bollwahn & Meermeier, 1989). Milk samples were, when possible, collected from sows suffering from CGM, and revealed a bacterial flora mainly comprising of *Streptococcus* spp. and *Staphylococcus* spp. (Paper V, Tables 4a and 4b). Attempts to isolate bacteria from tissue specimens collected from the same glands as were sampled for milk, failed regarding the *Streptococcus* spp. but were successful for the *Staphylococcus* spp. Bacteriological examination of tissue specimens severely affected by CGM, revealed a flora comprising the same species as obtained from the milk secretion collected 7 days after weaning (Paper IV). Furthermore, other bacteria such as *Peptostreptococcus anaerobicus*, *Propionibacterium granulosum* and *Prevotella* spp. were also isolated. All the aerobic and anaerobic bacteria were almost always yielded in mixed floras. The bacterial flora isolated in the present studies, (Papers IV, V, Thesis p. 34), with special reference to the tissue, are in agreement with other studies of mammary glands with abscesses or CGM (Englert et al., 1956; Delgado & Jones, 1981; Bollwahn & Meermeier, 1989; Pierskalla et al., 1990). It seems obvious that many different bacterial species are incriminated in the etiology of CGM and ubiquitously occur in the environment of sows (Ehrlich, 1912; Magnusson, 1928; Delgado & Jones, 1981; Pierskalla et al., 1990). The same flora of bacteria are commonly found in connection with other supperative lesions in the pig (McCracken & McCaughey, 1973; Jones, 1980; Engvall & Schwan, 1983).

Franke (1973a,b) isolated 9 microaerophilic strains of actinomycetes from 12 actinomycotic mammary glands of sows and suggested the name '*Actinomyces suis*'. '*Actinomyces suis*' has been isolated from the tonsils of pigs (Oomi et al., 1994; Patschimasiri et al., 1994). Oomi et al. (1994) claimed that the strain was morphologically and biologically comparable with the description of Franke and was transmitted from the piglets to the mammary glands of the sow by injuries or incisions caused by the sucking. Yamini & Slocombe (1988) reported that '*Actinomyces suis*' caused sporadic abortions in sows. The authors pointed out that anaerobic species such as *Actinomyces* are part of the normal flora of the mouth. More bewilderment arose when Ludwig et al. (1992) proposed that *Eubacterium suis* should be transferred to the genus *Actinomyces* as *Actinomyces suis* comb. nov. According to Ludwig et al. (1992), no type strain of the species *incertae sedis* '*Actinomyces suis*' Franke has been validly described. In our study, the strain of *Actinomyces* (Actinomyces - EF Group 78, CCUG 37626, Thesis p. 34) isolated from one CGM mammary gland, which also yielded growth of aerobic *Staphylococcus aureus* and *Streptococcus suis*, might indicate synergistic mechanisms between different aerobic and anaerobic bacterial species. Before this 'new' *Actinomyces* sp. will be accepted, phylogenetic affiliations of the species are certainly required. Nonetheless, the finding might act as encouragement for further elucidative studies in this particular anaerobic field of bacteriology.

In our study, histopathological examinations of severely destroyed mammary glands, (10/11 sows), fulfilled the criteria strongly associated with CGM in sows (Magnusson, 1928; Englert, 1961; Renk, 1962; Berger, 1969). In a study by Pierskalla et al. (1990) bacteriological examination and histopathological analysis failed to classify one-third of the mastitis with abscess formation as CGM.

Monitoring of the cell content of milk has been performed to quantify the influence of an environmental faecal flora on the inflammatory response in mammary glands at the time of parturition (Bertschinger et al., 1990). A similar approach might be applicable at the time of weaning (Papers IV, V). The TCC tended to be higher in milk from mammary glands with teat injuries and palpatory changes than in milk from clinically normal mammary glands (Paper IV, Table 7). A significant difference regarding the number of PMNLs and an almost significant difference regarding the percentage of PMNLs was also observed between the two clinical groups. The level of TCC in milk from clinically normal glands exceeded levels of cells reported at a comparable time of lactation (Drendel, 1991), or 1-2 weeks earlier (Wegmann, 1985; Magnusson et al., 1991), while the level of PMNLs in milk from clinically normal glands corresponded fairly well with those reported by Magnusson et al. (1991). There might be several explanations of the elevated TCC in the present studies. The increase in TCC and PMNLs in milk from clinically abnormal teats and glands compared with milk from clinically normal glands might reflect an inflammatory response caused by a temporary bacterial invasion of the mammary tissue. The successive increase in the ungentle sucking habit performed by the growing piglets might also have contributed to the elevated TCC in milk from clinically normal glands at the time of weaning. It has been suggested (Lee et al., 1983; Magnusson et al., 1991) that the increase in both

the number and percentage of epithelial cells recorded during the lactation is caused by the aggressive sucking behaviour of the growing piglets. As has been hypothesised previously, this sucking behaviour might also have been of considerable importance in the etiology of the teat injuries. In a broader sense, the observed increase in TCC might to some extent reflect an initial cessation in milk production, although the percentage of PMNLs recorded did not tend to increase, as has been previously shown concomitantly with discontinued sucking (Wegmann, 1985) or during the first days after weaning (Lee et al., 1983). The TCC and PMNLs showed a substantial numerical elevation in milk collected both from clinically normal and abnormal mammary glands within sows suffering from CGM (Paper V, Table 5). Obviously, the increase in TCC and PMNLs in milk from abnormal mammary glands reflected the presence of an inflammatory process, while the increase in cell parameters in milk from clinically normal mammary glands, might be speculated to be explained by influences from severe inflammatory processes in glands (CGM) within the same udder. More information is certainly needed about the average cell content in milk from clinically normal mammary glands at the time of weaning.

Conclusions

The following conclusions were drawn from 3 studies, (1) the influence of two feeding regimes, restricted versus standard, during the final 2 weeks of gestation on the clinical health status and performance of sows, with special reference to APP and udder health during the first 3 weeks of lactation and (2) the udder health of sows at time of weaning and 7 days after weaning with special focus on teat injuries and palpable changes of the mammary tissue and (3) chronic granulomatous mastitis (CGM) in sows.

- The occurrence of APP was significantly lower in the restricted fed (14.4%) than in the standard fed sows (26.6%). Irrespective of feeding regimes, APP sows revealed a significant increase in rectal temperature already one day before parturition and delivered a significantly higher number of stillborn piglets per litter (0.4), indicating an establishment of the disease already before parturition.

- The mean of the TCC values below the threshold of $10x10^6$ cells/ml in bacteria-free colostrum (day 1) and milk collected from clinically healthy sows increased significantly from day 1 to day 3 ($1.23x10^6$ vs $1.86x10^6$) and thereafter decreased gradually to day 22. The percentage of PMNLs decreased significantly from day 1 to day 3 (58.5% vs 49.0%), and thereafter remained on levels between 42% and 50%. Neither of the two feeding regimes influenced the TCC, the PMNLs or the pH in bacteria-free milk from clinically healthy sows.

- Bacteriological examinations of colostrum and milk from APP sows or from clinically healthy sows, yielded growth of *E. coli* from one or several mammary glands in approximately 80% and 30% of the lactations, respectively. The growth of *E. coli* declined until day 3 and was finally eliminated between days 3 and 8. Healthy sows with *E. coli* were designated as being subclinically infected. *E. coli* in pure cultures with a concomitant TCC exceeding $10x10^6$ cells/ml already on the first day of sampling occurred in 64% of the APP sows and in 16% of the clinically healthy sows. The TCC and PMNLs tended to be significantly higher in subclinical *E. coli* infected glands than in clinical *E. coli* infected glands (APP sows) ($157x10^6$ vs $82x10^6$ and 79.0% vs 65.8%, respectively). This might indicate a higher recruitment of cells to the subclinical *E. coli* infected glands.

- The TCC in bacteriologically negative colostrum was significantly higher in APP sows, than in clinically or subclinically *E. coli* infected sows, possibly being a result of a transient hypogalactic condition in milk production in APP sows. In contrast, the percentage of PMNLs was significantly higher in colostrum from clinically healthy sows than in the clinical or subclinical *E. coli*

infected sows. This might indicate that PMNLs have been redistributed to glands where they are required in the elimination of the pathogens.

- In sows with clinical or subclinical *E. coli* mastitis, only 38 (44%) of 87 *E. coli* mastitic glands (TCC>$10x10^6$ cells/ml) were revealed of the clinical examination. This indicates that clinical examination of the mammary glands post partum must be combined with bacteriological and cytological analyses of colostrum and milk before any further interpretations are made.

- The teat- and udder skin injuries were numerically more frequent within the two thoracic and first three abdominal teat pairs, varying from 11% to 16%. A large number of mammary glands were in full milk production. Presumably, there is a link between the vigorous sucking behaviour of the piglets and the origin of the injuries. The clinical appearance of the teat injuries subsided and at re-examination 7 days after weaning, 76% of the investigated teats were scored as normal.

- The prevalence of palpable changes at weaning increased gradually from the first to the second and subsequent lactations (15%, 30% and 60%). The observations might indicate a negative impact on the udder health with increasing lactation number.

- Bacteriological examination of milk from clinically normal and abnormal glands and teats at weaning yielded growth of non- as well as α- and β- haemolytic streptococci. The β- haemolytic streptococci were more frequently isolated from abnormal glands and teats. A limited number of secretion samples obtained 7 days after weaning yielded growth of staphylococci and *A. pyogenes*.

- A cytological comparison of milk from clinically normal and abnormal mammary glands and teats revealed almost significantly or significantly increased levels of TCC and PMNLs in the milk from abnormal glands and teats. The increase in TCC and PMNLs in milk from these glands reflect a defence reaction caused by a temporary bacterial invasion of the mammary glands.

- Chronic granulomatous mastitis is the ultimate and most severe consequence of infectious mastitis at time of weaning. Ten sows out of 11, that had been clinically examined before slaughter, were histopathologically confirmed with the diagnosis of CGM after slaughter.

- The bacterial flora isolated from mammary gland tissue affected by CGM, and often not possible to sample for milk, comprised *A. pyogenes*, *F. necrophorum*, *S. aureus*, *P. anaerobicus*, *P. granulosum* and *Prevotella* spp.

- Bacteriological examination of mammary tissue from an additional 3 sows affected with CGM demonstrated that other *Actinomyces* spp. (Actinomyces - EF Group 78, CCUG 37626), might also be of great importance in the etiology of the CGM. The growth of the *Actinomyces* spp. might be promoted by the other bacterial flora isolated. A potential linkage might exist between vigorously sucking piglets causing teat- and udder skin injuries and the transmission of these bacteria.

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Sir Winston Churchill (1874-1965)

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