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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

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Persson, A., A. Pedersen Mörner and W. Kuhl: A long term study on the health status and perfomance 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. Acta vet. scand. 1996, 37, 279-291. – The main objective of this study was to determine the total cell content, TCC, and the percentage of polymorphonuclear leucocytes, PMNLs, in colostrum and milk collected from sows during the first 22 days of lactation. The pH-values during the same sampling period were also determined. It should be emphasized that all the values obtained emanate from bacteriologically negative colostrum and milk. The potential influence of different levels of late gestation feeding regimes was also evaluated.

The TCC-values obtained from milk samples during the first 3 weeks of lactation and exceeding the designated threshold of 10×10^6 cells/ml varied between 4% and 21%. Within the TCC-limitation of 10-19.99$\times10^6$ cells/ml neither the preceding nor the succeeding cell counts exceeded the threshold in 26.8%. TCC-values above 19.99$\times10^6$ cells/ml were preceded and succeeded by cell counts below the threshold in 58.8% and 58.8%, respectively.

The TCC-levels below the threshold of 10×10^6 cells/ml, expressed as geometric least square means, increased significantly from day 1 to day 3 (1.23×10^6 cells/ml versus 1.86×10^6 cells/ml) and decreased thereafter gradually to day 22 (1.38×10^6 cells/ml). When all values were included, the TCC-values increased in a similar pattern from day 1 to day 3 (1.38×10^6 cells/ml versus 3.18×10^6 cells/ml). The value on day 22 of lactation was still on a significantly elevated level compared with that of day 1 (2.10×10^6 cells/ml versus 1.38×10^6 cells/ml).

The 2 different feeding regimes were not found to influence the TCC-values during the first 22 days of lactation.

In the whole material the PMNL-values, expressed as percentages of the TCC, declined from approximately 60% on day 1 of lactation to between 40% and 50% for the remaining sampling period. This decline was comparable with the one seen in the cell class below the threshold of 10×10^6 cells/ml. In the 2 cell classes above 9.99×10^6 cells/ml, 78.0% and 88.8% of PMNLs on day 1 declined to about 40% on day 22. This might indicate an inflammatory response on day 1 but without any detectable bacteriological growth.

The increase in lactation number, if lactation 1 was compared with the following lactations, revealed a significant rise ($p<0.05$) in TCC-level and percentage level of PMNLs.

A stepwise and significant increase in pH-level occurred between days 1, 3 and 8 (6.18, 6.56, 7.03) followed by a significant decrease to day 22 (6.91) when pH-values from milk of all cell classes were included.

agalactia; post partum; feeding; lactation; pH.

Introduction

The total cell content per volume unit, TCC, and its different cellular components, in colostrum and milk samples from post parturient sows, were identified and described in some earlier studies *(Evans et al.* 1982, *Lee et al.* 1983, *Wegmann* 1985, *Schollenberg et al.* 1986, *Hurley & Grieve* 1988, *Magnusson et al.* 1991). In these 6 studies, milk samples were collected during the first 21-31 days of lactation and in all of them the proportion of polymorphonuclear leucocytes, PMNLs, of the TCC was of fundamental interest. The authors, with the exception of *Wegmann* (1985), considered the PMNLs to be the most prominent cell in colostrum and to possess the ability to rapidly defend the udder glands against infection. The preparation of the cells in the colostrum and milk differed considerably between the studies. The TCC per ml colostrum or milk varied considerably in these studies with a range from a few hundred thousands up to 10 millions and an approximate average value of one to 2 million cells per ml. The percentage of PMNLs varied in colostrum (day 1) between 56-72, thereafter gradually decreasing during the lactation to percentages from 30 to 40. *Wegmann* (1985) reported divergent results, proposing that the PMNLs seldom reached more than 10 percent in milk from suckled mammary glands.

Faith et al. (1979) reported that the PMNLs and macrophages, approximately 45% and 50% respectively, were the predominant cells in the colostral secretion from women. The PMNLs, identified by using electron microscopy, were also considered to be the principal cells in colostrum in cows *(Lee et al.* 1980). In this study, the PMNLs varied between 50% and 80% of the total cell count and were reported to increase in proportion during the colostral period, in the absence of any detectable infection.

To our knowledge no long-term study on the cell content in colostrum and milk, comprising several consecutive lactations, has been performed in sows. The sows in the present study were fed according to 2 different regimes during late pregnancy. Their influence on health performance after farrowing was described earlier *(Persson et al.* 1989). The experimental design allowed us to study the influence that may possibly occur on the TCC and PMNLs.

The objective of this study was to determine the TCC and its proportion of PMNLs in bacteria-free colostrum and milk samples from clinically healthy sows. The sows were sampled on 4 predetermined days during the first 3 weeks postpartum, on days 1, 3, 8 and 22, in 6 consecutive lactations when possible. It was also our intention to elucidate the possible influence of the 2 different late pregnancy feeding regimes on the outcome of the TCC and PMNLs. The pH-levels were also measured in colostrum and milk samples.

Materials and methods

Animals and feeding

A total of 39 pairs of full sibs (Swedish Landrace×Swedish Yorkshire) were used in the experiment out of which 24 entered the trial as gilts. Each sow was then, if possible, kept for 6 parities.

One sow in each pair was randomly allocated to the control group and the other to the experimental group. Three weeks before expected farrowing the sows were transferred to the farrowing pens. All sows were kept loose during farrowing and lactation.

The animals in the control group, (C), were fed 2.4 kg/day for the first 100 days of pregnancy and then 3.4 kg/day from day 100 of gestation until farrowing. The experimental group, (E), was fed 1.0 kg/day during the last 15 days of gestation. The experimental group was offered extra feed between days 30 and 100 of gestation. The total amount of feed given during the

gestation period was equalized for both groups. The composition of the feed was described by *Persson et al.* (1989). During lactation, the daily feed allowance was successively increased for all sows to a maximum of 4.0 kg + 0.2 per piglet within 14 days after farrowing.

Clinical examination and collection of colostrum and milk samples

The rectal temperature was monitored every morning and evening, starting 2 days before expected farrowing and continuing until 2 days after parturition. Sows with a temperature exceeding 39.5°C within 48 h after parturition were considered to be diseased (agalactia post partum) and were clinically examined and treated medically.

Colostrum samples were collected on the day of parturition, day 1, from all healthy sows farrowing during working hours. Piglets were removed from the sow 30 min before milking. The udder was washed with soap solution and warm water followed by careful disinfection with iodine and 70% alcohol. Oxytocin was given i.m. (20 IU) to promote milk let-down. The 2 first streams of secretion were discarded from both canals of the teat. Approximately 0.5 ml secretion was collected for bacteriological examination followed by an additional 2 ml for cytological analysis. At least 4 glands of each sow were milked. The colostrum and milk samples from agalactia post partum sows were collected from mammary glands with clinical signs of mastitis such as hardening, swelling and erythema. Results from this material are not included in this presentation. The sampling procedure was repeated 2, 7 and 21 days after the first sampling. 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 (17% of all samples). Colostrum and milk samples were collected during altogether 96 lactations of clinically healthy sows.

Cell count

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 0.5×2 cm (1 sq.cm) 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 count and differential counts of polymorphonuclear leucocytes were carried out at a magnification of ×1000 under oil in a light microscope.

Bacteriological examination

With a calibrated plastic inoculating loop (A/S Nunc, Roskilde, Denmark), 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 incubation for 24 h at 37°C. In addition, duplicate blood agar plates were incubated anaerobically for 24 h at 37°C in BBL Gas Pak® System Oxoid Gas Generating Kit (Oxoid Ltd., Hampshire, England).

Determination of 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).

Classification of colostrum and milk samples according to clinical status, bacteriology and cell content

Before the statistical analysis was performed the bacteriologically negative colostrum and milk samples were divided into 2 groups according to the health status of the sows. The sows were first clinically classified as diseased or healthy. When bacteriological culturing was performed from colostrum and milk samples collected from apparently healthy sows, a subgroup revealed growth of *E. coli* in pure cultures and with a TCC exceeding 10×10^6 cells/ml on the first or second sampling occasion from one or more mammary glands. This group was denoted as being "subclinically infected sows". The result from this material is not included in this presentation. (For further details reference should be made to manuscript number III in this series.)

The statistical analyses were performed on all the TCC data from clinically healthy sows and also on 3 different sub-sets, according to the TCC-level in the colostrum and milk sample; TCC = $0\text{-}9.99\times10^6$, TCC = $10\text{-}19.99\times10^6$ and TCC>19.99×10^6. The lowest cell class limit was established from bacteriologically negative colostrum and milk samples, by calculating the mean ± 2 SD, and was found to be close to 10×10^6 cells/ml. The results concerning the pH-values derived from the whole dataset and also from one sub-set; TCC = $0\text{-}9.99\times10^6$.

It should be emphasized that the results obtained in this presentation are from bacteriologically negative mammary glands in clinically healthy sows in spite of the analyses of variance including all 3 categories of sows; clinically healthy sows with or without subclinical E. coli infection, and sows clinically diseased (agalactia post partum). These clinically healthy sows were also free from subclinical infection of *E. coli* with a concomitant TCC exceeding 10×10^6 cells/ml colostrum and milk.

Statistical methods

Statistical evaluation was performed using analysis of variance (GLM procedures) available from the Statistical Analysis System *(SAS Institute Inc.* 1985).

The response variables – TCC, PMNLs and the pH-value – were analysed according to different statistical models.

Model 1: The effect of feeding and the effect of health status of the sows and the interaction between these 2 effects. Feeding was represented by 2 classes, restricted (E), versus high feeding level (C). Health status was represented by 3 classes; clinically healthy sows; clinically healthy but subclinically infected sows, and clinically diseased sows (agal. post part.).

Model 2: The effect of day of sampling (4 classes, days 1, 3, 8 and 22 after farrowing), the effect of health status of the sows (3 classes; see the health status as designated above) and the interaction between these 2 effects.

Model 3: The effect of lactation number (6 classes; lactation 1 to 6), the effect of health status of the sows (3 classes; see above) and the interaction between these 2 effects.

Model 4: The variation in TCC-values within sow was tested. Within health status, day of sampling and sow, the variance in TCC was calculated (in a total of 97 observations from 41 sows). This value was analysed by analysis of variance according to a model including the effect of health status (3 classes) and day of sampling.

Using the analysis of variance requires that the observations are normally distributed. Since the variable TCC is far from normally distributed, these values were transformed by using logarithmic transformation before statistical evaluations were performed. The statistical tests are presented directly from obtained results while

Table 1. The Total Cell Content, TCC, according to day of lactation, in bacteriologically negative colostrum and milk, collected from mammary glands in clinically healthy sows. The TCC-values ($\times 10^{-6}$/ml) are presented as geometric means with a 95% confidence interval. The statistical analyses were performed after logarithmic transformation and the statistical presentation emanates from these calculations. Numbers of glands sampled are given in parentheses ( ), the number of corresponding sows are given in square brackets [ ].

Day of lactation	Total Cell Content			All TCC-values
	0-9.99	10-19.99	>19.99	
1	1.23 (1.08-1.39)[a] (239) [37]	14.78 (12.31-17.74) (4) [4]	27.29 (15.95-46.68) (6) [4]	1.38 (1.15-1.65)[a] (249) [37]
3	1.86 (1.60-2.16)[b] (166) [35]	13.19 (12.20-14.26) (22) [15]	43.74 (33.05-57.90) (22) [12]	3.18 (2.61-3.86)[b] (210) [35]
8	1.53 (1.29-1.81)[bc] (129) [33]	12.54 (10.65-14.76) (5) [5]	60.71 (43.23-85.25) (15) [9]	2.37 (1.88-2.99)[bc] (149) [34]
22	1.38 (1.14-1.68)[ac] (100) [29]	12.16 (10.83-13.65) (10) [9]	44.53 (27.97-70.89) (8) [5]	2.10 (1.62-2.72)[c] (118) [31]

Mean values within a column without a superscript letter in common differ significantly ($p<0.05$).

the numerical values of the TCC are presented as antilogarithmics of least-square means for the log-values (later called geometric mean) with a 95% confidence interval within parenthesis (Table 1).

Results

The Total Cell Content, TCC, in bacteriologically negative colostrum and milk, collected from mammary glands in clinically healthy sows

The results of the TCC are described in Table 1. The majority of the TCC values obtained are below a threshold of 10×10^6/ml in colostrum or milk. Nevertheless, a minor fraction exceeded the abovementioned threshold: 10 out of 249 on day 1, 44 out of 210 on day 3, 20 out of 149 on day 8 and finally 18 out of 118 on day 22 of lactation.

The geometric mean of the TCC values below the threshold of 10×10^6 cells/ml (Table 1) increased significantly from day 1 to day 3 (1.23 versus 1.86) ($p<0.001$) and decreased thereafter gradually to day 22 ($p<0.05$).

The results when all TCC-values were included in the analysis (Table 1) agreed with the pattern for the TCC values below the threshold value of 10×10^6 cells/ml. Thus, the geometric mean of the TCC-values on day 1, significantly increased to day 3 (1.38 versus 3.18) ($p<0.001$). The decrease of TCC from day 3 to day 8 and from day 8 to day 22 was not significant, while the decrease from day 3 to day 22 was significant ($p<0.05$). The geometric mean value of TCC on day 22 was still on a significantly elevated level compared with that on day 1 (2.10 versus 1.38) ($p<0.01$).

When the variance in TCC-values between mammary glands within the same sow was analysed, no significant variation could be revealed. It should be emphasized that these calculations were performed on data from 41

Table 2. TCC-values below 10×10^6 cells/ml in samples preceding or succeeding the 2 cell classes denoted 10 – 19.99×10^6 cells/ml or $>19.99\times10^6$ cells/ml. The samples from the 2 cell classes were obtained from bacteriologically negative colostrum and milk in clinically healthy sows. Number of samples included are presented in parentheses.

	Total Cell Content	
	10-19.99	>19.99
% of preceding TCC-values being below 10×10^6 cells/ml.	63.4% (26)	58.8% (30)
% of succeeding TCC-values being below 10×10^6 cells/ml.	46.3% (19)	58.8% (30)
% of TCC-values where both the preceding and the succeeding TCC were below 10×10^6 cells/ml.	26.8% (11)	17.6% (9)

sows, and only from observations below the threshold of 10×10^6 cells/ml.

Data in Tables 1 and 2 show that values within the TCC-limitation of $10\text{-}19.99\times10^6$ cells/ml were preceded by cell counts below the threshold of 10×10^6 in 63.4% and followed by cell counts that were also below the threshold in 46.3%. In 26.8% neither the preceding nor the succeeding cell counts exceeded the threshold. The TCC-values above the limit of $>19.99\times10^6$ cells/ml were preceded and succeeded by cell counts below the threshold in 58.8% and 58.8%, respectively. A majority of the TCC-values (Table 1), above 10×10^6 cells/ml, were obtained from colostrum and milk samples emanating from a limited number of sows. Each of these sows contributed with 2-6 TCC-values.

The 2 different feeding regimes were not found to influence the TCC-values during the first 22 days of lactation.

Table 3. The PolyMorphoNuclear Leucocytes, PMNLs, expressed as percentage of the TCC, and presented according to cell class and day of lactation. The collection of bacteriologically negative colostrum and milk samples was from mammary glands in clinically healthy sows. The PMNL percentage values are presented as least-square mean ± S.E.M. Number of glands sampled are given in parentheses ( ), the number of corresponding sows are given in square brackets [ ].

Day of lactation	Total Cell Content			All TCC-values
	0 - 9.99	10 - 19.99	>19.99	
1	58.5 ± 1.6^a (237) [37]	78.0 ± 15.0 (4) [3]	88.8 ± 12.0 (6) [4]	59.6 ± 1.7^a (247) [37]
3	49.0 ± 2.0^b (164) [35]	44.3 ± 6.5 (21) [14]	54.9 ± 6.3 (22) [12]	49.1 ± 1.8^b (207) [35]
8	42.2 ± 2.2^c (126) [33]	23.5 ± 13.4 (5) [5]	61.5 ± 7.6 (15) [9]	43.6 ± 2.2^b (146) [34]
22	50.0 ± 2.6^b (94) [29]	45.8 ± 9.5 (10) [9]	38.6 ± 11.1 (7) [5]	48.9 ± 2.5^b (111) [31]

Percentage values within a column without a superscript letter in common differ significantly ($p<0.05$).

Table 4. The Total Cell Content, TCC, and the PolyMorphoNuclear Leucocytes, PMNLs, in bacteriologically negative colostrum and milk, collected from mammary glands in clinically healthy sows throughout six consecutive lactations. The PMNLs are expressed as percentage of the TCC. The PMNL percentage values are presented as least-square mean ± S.E.M. The TCC-values ($\times 10^{-6}$/ml) are presented as geometric mean with a 95% confidence interval. The statistical analyses were performed on the original TCC-values after logarithmic transformation and the statistical presentation emanates from these calculations. Number of glands sampled are given in parentheses ( ), the number of corresponding sows are given in square brackets [ ].

Lactation number	TCC and PMNL % from cell class 0-9.99×10^6 cells/ml.	
	TCC	PMNL %
1	1.01 (0.82-1.23)^a (88) [10]	42.8 ± 2.6^a (88) [10]
2	1.45 (1.19-1.77)^{bc} (96) [8]	49.3 ± 2.6^{ab} (93) [8]
3	1.26 (1.03-1.53)^{ab} (95) [10]	53.0 ± 2.6^{bd} (93) [9]
4	1.85 (1.52-2.27)^c (92) [9]	55.1 ± 2.6^{bc} (91) [9]
5	1.78 (1.52-2.07)^c (159) [15]	49.7 ± 2.0^b (159) [15]
6	1.37 (1.13-1.65)^b (104) [10]	59.0 ± 2.5^{cd} (97) [9]

Mean values, regarding TCC and percentage of PMNLs, within a column without a small superscript letter in common differ significantly ($p<0.05$).

The lactation number had a significant influence on the TCC values below 10×10^6 cells/ml (Table 4). There was a significant increase in TCC ($p<0.05$) when lactation 1 was compared with consecutive lactations 2, 4, 5 and 6. The TCC of the first lactation showed a geometric mean value of 1.01×10^6 cells/ml. The mean value of TCC reached a maximum in lactations 4 and 5 (1.85 versus 1.78), and decreased thereafter to 1.37×10^6 cells/ml in the final lactation, number 6.

The PolyMorphoNuclearLeucocytes, PMNLs, in bacteriologically negative colostrum and milk, collected from mammary glands in clinically healthy sows

The PolyMorphoNuclearLeucocytes, PMNLs, are expressed as their percentage share of the TCC (Table 3). Below the threshold value, 10×10^6 cells/ml the percentage of PMNLs declined significantly ($p<0.001$) from 58.5% on day 1 to 49.0% on day 3 of lactation. Thereafter, the percentage of PMNLs further decreased

Table 5. A comparison between pH-values according to day of lactation and deriving from bacteriologically negative colostrum and milk collected from mammary glands in clinically healthy sows. The pH-values are given as least-square mean ± S.E.M. Number of glands sampled are given in parentheses ( ), the number of corresponding sows are given in square brackets [ ].

Day of lactation	pH-values from different TCC – classes	
	0 – 9.99	All TCC – values
1	6.17 ± 0.01[a] (189) [30]	6.18 0.01[a] (199) [30]
3	6.55 ± 0.02[b] (83) [20]	6.56 0.02[b] (100) [22]
8	7.01 ± 0.03[c] (49) [17]	7.03(0.03[c] (56) [19]
22	6.86 ± 0.03[d] (39) [16]	6.91(0.03[d] (46) [17]

pH-values within a column without a superscript letter in common differ significantly. In first column ($p<0.001$) and in the second column ($p<0.01$).

to 42.2% on day 8 ($p<0.05$) followed by an increase to 50.0% on day 22 ($p<0.05$).

When the PMNL-analysis included all the TCC-values, the percentage of PMNLs decreased significantly from 59.6% on day 1 to 49.1% on day 3 ($p<0.001$). No further significant change of the percentage of PMNLs occurred from day 3 to day 8 or from day 8 to day 22.

The percentage values of PMNLs, derived from the TCC-values exceeding 19.99×10^6 cells/ml, were higher than the PMNL-values emanating from the TCC-values below 10×10^6 cells/ml on days 1, 3 and 8 (Table 3).

Neither of the 2 feeding regimes influenced the percentage level of PMNLs on the consecutive 4 sampling occasions during the first 3 weeks of lactation.

Within the cell class below 10×10^6 cells/ml, the PMNL percentage increased from the first lactation, 42.8% ($p<0.05$), to the successive lactations 3 to 6 (Table 4). In lactations 2 to 5 the PMNLs fluctuated between 49.3% and 55.1%. This tendency was approximately the same even if the calculation was based on all 3 cell classes, with the exception that no significant difference was found between lactations 1 and 5.

A comparison between pH-values in bacteriologically negative colostrum and milk in clinically healthy sows

A comparison between pH-values deriving from bacteriologically negative colostrum and milk samples is presented in Table 5. The pH in the colostrum and milk with a total cell content below the threshold value, increased from 6.17 to 6.55 between days 1 and 3 and further to 7.01

on day 8 ($p<0.001$). From day 8 to day 22 there was a significant decline of the pH from 7.01 to 6.86 ($p<0.001$).

The pH-values from colostrum and milk samples remained stable even when the pH-values deriving from milk with cell counts exceeding 10×10^6 cells/ml were included. The changes between sampling days were almost at the same level as described previously.

The 2 different late pregnancy feeding regimes did not influence the pH.

Discussion

The objectives of this long-term study were to determine the total cell content, TCC, its percentage of polymorphonuclear leucocytes, PMNLs, and pH in bacteriologically negative colostrum and milk from clinically healthy sows. To our knowledge, no results from a long-term experiment conducted under strictly standardized conditions with the same sows have been reported previously.

The 2 different feeding regimes imposed during gestation had no influence on the outcome of the TCC-values, the percentage of PMNLs or the pH.

The lactation number had a significantly increasing influence on the TCC and the percentage of PMNLs when the calculations were performed from the cell class below 10×10^6 cells/ml. Thus there was a significant increase in TCC from lactation 1 to lactations 2, 4, 5 and 6. The percentage level of PMNLs also increased from lactation 1 to lactations 3-6. These observations might indicate an increasing stimulus on the sow's immune system with elevated lactation number. In cows, *Brolund* (1985) and *Emanuelsson* (1984) stated that there was a systematic increase in base-level cell counts with lactation number. Several factors were suggested to be involved, among them physiological age differences and effects of previously eliminated infections *(Brolund* 1985). In the cow, *Blackburn* (1966) also found that the average total cell count increased in consecutive lactations up to the seventh. This rise was attributable to the increase in number of polymorphs. In the present study, although based on a rather limited number of observations, the percentage of PMNLs in colostrum and milk rose significantly from the first to the 4th lactation and thereafter stabilized at levels of 50 to 60%.

The geometric mean value of all TCC-values on day 1 of lactation, 1.38×10^6 cells/ml colostrum, was higher compared with the earlier reported observations presented in logarithmic terms *(Hurley et al.* 1988). The total cell content, reported from other studies *(Ross et al.* 1981, *Evans et al.* 1982, *Magnusson et al.* 1991) was demonstrated to be higher on day 1 than in the present study. The increase in TCC from day 1 to day 3 , although on a slightly higher level, is supported by previous investigations *(Wegmann* 1985, *Schollenberg et al.* 1986). During the subsequent sampling period, days 3 to 22, especially with regard to the cell class below 10×10^6/ml, the geometric mean values of TCC were characterized by a stepwise decrease, well in accordance with earlier reports *(Wegmann* 1985, *Schollenberg 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 the milk production might have contributed to a slight dilution effect of the TCC already starting after day 3. In women, after 2 weeks of lactation, the total cell content continues to fall while milk volume increases *(Faith et al.* 1979). In the cow, the early stage of lactation, more precisely the first 2 weeks, was distinguished by declining somatic cell counts according to *Cullen et al.* (1968). This report was based on data from bacteriologically negative foremilk sam-

ples. *Kennedy et al.* (1982) and *Emanuelsson et al.* (1984) pointed out that the lowest somatic cell counts in the cow coincided fairly closely with peak lactation. Further, 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.

The calculation and the establishment of a certain upper limitation of the TCC, 10×10^6 cells/ml, which indicates an inflammatory response when it is exceeded, may be a question for further research. In an early stage, during very preliminary calculations of the mean value, we decided to settle the threshold approximately 2 standard deviations above the estimated mean. *Bertschinger et al.* (1990) based upon earlier studies *(Wegmann* 1985), decided to place the threshold at 5×10^6 cells / ml when the PMNL content exceeded 70%. The percentage of PMNLs was not a determining factor when the threshold was settled in our study. It could, in addition, be demonstrated that the percentage of PMNLs, on an average, did not reach 70% of the total cell content before the limitation of 10×10^6 cells/ml was exceeded on day 1 of lactation.

In the present study, between 4 and 21% of the TCC-values, depending on the sampling day, exceeded the designated threshold of 10×10^6 cells / ml. The TCC-values might have been influenced by the fact that colostrum and milk derive from 2 separate duct systems within the mammary gland *(Elmore & Martin* 1986). One separate segment of the mammary gland can be partially inflamed or completely atrophied. To some extent, this contradicts the findings in our study, where almost 50% of the subsequent TCC-values are below the threshold on the next sampling occasion. In approximately 25% of the elevated TCC-values, neither the preceding nor the subsequent cell counts exceeded the threshold. A possible explanation of these temporarily exceeded TCC-values might be that the 2 segments of the mammary glands are irregularly emptied (are not suckled regularly and completely).

The polymorphonuclear leucocytes were the predominant cells in colostral secretion, approximately 60%, regardless whether the calculations were done using counts below 10×10^6 cells/ml or from all the values. These results are well in accordance with results from some earlier studies *(Lee et al.* 1983, *Schollenberger et al.* 1986, *Hurley & Grieve* 1988, *Magnusson et al.* 1991). *Evans et al.* (1982) found a slightly higher percentage of PMNLs (71.7%), in colostral secretion, which could also be compared with the situation in women *(Faith et al.* 1979), where the PMNLs together with the macrophages are the predominant cells in the colostral secretion. The PMN leucocytes were also considered to be the principal cell in colostral secretion in cows *(Lee et al.* 1980) and were reported to increase in proportion during the colostral period in the absence of any detectable infection. As has been stated earlier in this discussion, the PMNL-level in sows did not exceed 70% until estimations were performed from the cell classes above 10×10^6 cells/ml. This elevation in percentage of PMNLs was only found on the first sampling occasion post partum. It must also be emphasized that this noticeable increase in percentage of PMNLs was uninfluenced by any detectable bacteriological growth. *Wegmann* (1985), on the other hand, reported that the proportion of PMNLs was below 10% or impossible to count in suckled teats. The proportion of PMNLs was only elevated in milk secretion collected from unsuckled teats or from mammary glands with mastitis.

There was a pronounced decline in percentage of PMNLs, from approximately 60% on day 1 to 50% or less during the remaining sampling period. *Schollenberger et al.* (1986) described an almost identical course. *Evans et al.* (1982)

and *Lee et al.* (1983) registered a somewhat more pronounced decrease around day 20 of lactation. It has been suggested *(Magnusson et al.* 1991) that during the time period after day 1 of lactation there is a decline of PMNLs to between 14 and 33%, the epithelial cells being predominant during this period.

The pH showed a pronounced increase during the first week of lactation as compared with the rest of the sampling period. This increase was highly significant. The numerically small, but nevertheless significant, decrease from day 8 to day 22 is also of interest. Even if all cell classes were taken into account, the pH did not change. The results obtained by *Martin et al.* (1967), who showed that pH from normal sow's colostrum and milk, 1-3 days post partum, ranged from 6.0 to 6.9 (average 6.6) are consistent with our results. *Ringarp* (1960) measured the pH of milk in 248 clinically healthy sows with apparently normal lactation 12 to 72 h post partum. In 94.4% of the sows these pH-values ranged from 6.4 and 6.5, the measurements being performed by means of a special pH-indicator paper. *Ross et al.* (1981) surprisingly obtained slightly higher pH-values in milk secretion from normal mammary glands than from samples collected from mastitic glands. He claimed that the results might have been influenced by the fact that mammary glands could contribute with milk from both normal and less severely affected segments mixed with secretion from mastitic ones.

Conclusions

The aim of this study was to describe alterations in the TCC, its percentage of PMNLs and the pH in colostrum and milk from clinically healthy sows during 4 consecutive sampling occasions during the first 3 weeks of lactation. The significant increase in TCC between days 1 and 3 was followed by a decrease to day 22. The percentage of PMNLs exhibited an evident decline from day 1 to day 3, and thereafter fluctuated on a stabilized level. The pH exhibited a pronounced increase during the first week of lactation and thereafter remained on approximately the same level.

The 2 different feeding regimes to which the sows were allotted, did not seem to influence either the TCC, the percentages of PMNLs, or the pH.

To some extent the lactation number revealed an influence on an increasing TCC and an increasing percentage of PMNLs if parity 1 was compared with the higher lactation numbers.

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References

Allen AD, Lasley JF: Milk production of sows. J. Anim. Sci. 1960, *19,* 150-155.

Bertschinger HU, Bürgi E, Eng V, Wegmann P: Senkung der Inzidenz von Puerperaler Mastitis bei der Sau durch Schutz des Gesäuges vor Verschmutzung. (Reduction of the incidence of puerperal mastitis in the sow by protection of the mammory gland against faecal contamination). Schweizer Arch. Tierheilk. 1990, *132,* 557-566.

Blackburn PS: The variation in the cell count of cows milk throughout lactation and from one lactation to the next. J. Dairy Res. 1966, *33,* 193-198.

Brolund L: Cell counts in bovine milk. Causes of variation and applicability for diagnosis of subclinical mastitis. Thesis, Swedish University of Agricultural Sciences, Uppsala. Acta vet. scand. 1985, Suppl. 80, 1-123.

Cullen GA: Cell counts throughout lactation. Vet. Rec. 1968, *83,* 125-128.

Elmore RG, Martin CE: Mammary glands. In: Leman A.D., Straw B., Glock R.D., Mengeling W.L., Penny R.H.C. and Scholl E. (ed.): Diseases of Swine, 1986, 6th edition. The Iowa State University Press, Ames, Iowa, U.S.A., pp 168-183.

Emanuelsson U, Persson E: Studies on somatic cell counts in milk from Swedish dairy cows. I. Non-genetic causes of variation in monthly test-day results. Acta agric. scand. 1984, *34,* 33-44.

Evans PA, Newby TJ, Strokes CR, Bourne FJ: A study of cells in the mammary secretions of sows. Vet. Immun. Immunopath. 1982, *3,* 515-527.

Faith CSH, Rosamond LCW, Lawton JWM: Human colostrum and breast milk cells. Acta paediatr. scand. 1979, *68,* 389-396.

Hurley WL, Grieve RCJ: Total and differential cell counts and N-acetyl-β-D-Glucosaminidase activity in sow milk during lactation. Vet. Res. Comm. 1988, 12, 149-153.

Kennedy BW, Sethar MS, Tong AKW, Moxley JE, Downey BR: Environmental factors influencing test-day somatic cell counts in Holsteins. J. Dairy Sci.1982, *65,* 275-280.

Lee CS, Wooding P, Kemp P: Identification, properties, and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. J. Dairy Res. 1980, *47,* 39-50.

Lee CS, McCauley I, Hartman PE: Light and electron microscopy of cells in pig colostrum, milk and involution secretion. Acta anat. 1983, *117,* 270-280.

Magnusson U, Rodriguez-Martinez H, Einarsson S: A simple and rapid method using acridine orange for differential cell count in porcine mammary secretion. Vet. Rec. 1991, *129,* 485-490.

Martin CE, Hooper BE, Armstrong CH, Amstutz HE: A clinical and pathological study of the mastitis-metritis-agalactia syndrome of sows. J. Amer. vet. med. Ass. 1967, *151,* 1629-1634.

Persson A, Pedersen AE, Göransson L, Kuhl W: A longterm 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. Acta vet. scand. 1989, *30,* 9-17.

Prescott SC, Breed RS: The determination of the number of body cells in milk by a direct method. J. infect. Dis. 1910, *7,* 632-640.

Ringarp N: Clinical and experimental investigations into a post parturient syndrome with agalactia in sows. Acta agric. scand. 1960, Suppl. 7, 1-166.

Ross RF, Orning AP, Woods RD, Zimmermann BJ, Cox DF, Harries DL: Bacteriological study of sow agalactia. Amer. J. vet. Res. 1981, *42,* 949-955.

SAS Institute Inc: SAS user's guide. Statistics Version 5. Cary, N.C. 1985.

Schollenberger A, Degórski A, Frymus T, Schollenberger A: Cells of sow mammary secretion. I. Morphology and differential counts during lactation. J.Vet. Med. A 1986, *33,* 31-38.

Wegmann P: Zur Pathogenese der Colimatitis beim Mutterschwein. (Pathogenesis of coliform mastitis in the sow). Inaugural-Dissertation, Zürich 1985, 1-81.

Sammanfattning

En långtidsstudie rörande hälsotillstånd och produktionsresultat hos suggor på olika fodergivor under sen dräktighet.

II. Det totala cellinnehållet samt den procentuella andelen polymorfkärniga leukocyter i kolostrum och mjölk.

Undersökningen har till syfte att beskriva det totala cellinnehållet, TCC, och dess procentuella andel polymorfkärniga leukocyter, PMNL, i suggans kolostrum och mjölk vid fyra provtagningstillfällen under de första 3 veckorna av laktationen. pH bestämdes också i dessa sekret när så var möjligt. Modersuggorna undersöktes under 6 på varandra följande laktationer. Det bör speciellt påpekas att man vid bakteriologisk odling från mjölkproven inte kunde påvisa någon växt.

Trots negativt bakteriologiskt provsvar överskreds gränsvärdet 10×106 celler/ml i 4-21% av celltalsbestämningarna. Dessa höga celltalsvärden föregicks eller efterföljdes i ett flertal fall av värden som var lägre än det uppsatta gränsvärdet. De förhöjda celltalsvärdena tolkades som att juverdelen endast tillfälligtvis tillåtits avsina eller eventuellt varit utsatt för ett inflammatoriskt stimuli.

TCC i kolostrum och mjölk ökade signifikant mellan dag 1 och 3, från $1{,}23\times10^6$ celler/ml till $1{,}86\times10^6$ celler/ml och minskade därefter fram till dag 22 till $1{,}38\times10^6$ celler/ml. Motsvarande celltalsnivåer, när alla mätvärden inkluderades, uppmättes till $1{,}38\times10^6$, $3{,}18\times10^6$ och $2{,}10\times10^6$ celler/ml respektive. TCC på dag 22 ($2{,}10\times10^6$ celler/ml) var signifikant högre än dag 1.

Den procentuella andelen PMNL, uppgick till 60 på dag 1 och sjönk sedan signifikant till en nivå varierande mellan 40-50. Procentandelen PMNL förblev oförändrad oavsett om alla mätvärden inkluderades eller om man inskränkte analysen till värden erhållna ur gruppen $<10\times10^6$ celler/ml. Om man endast utförde beräkningar från mätvärden $>19,99\times10^6$ celler/ml , fann man att andelen PMNL uppgick till 88,8% på dag 1 och hade sjunkit till 61,5% på dag 8. De två olika utfodringsnivåerna, under senare delen av dräktigheten, tycktes inte ha haft någon påverkan på det totala cellinnehållet, TCC, i kolostrum eller mjölk eller på procentandelen PMNL under de första 3 veckorna av laktationen.

Med stigande laktationsnummer påvisades en signifikant stegring av TCC och procentandelen PMNL. Dessa signifikanta förändringar kunde utläsas som en ökning mellan laktation 1 och de därefter i nummerföljd kommande laktationerna.

pH-värdena i kolstrum och mjölk uppmätta ur gruppen "alla celltalsvärden" steg gradvis och signifikant mellan dag 1, 3 och 8; 6,18, 6,56 och 7,03 respektive. Dag 22 uppmättes pH till 6,91, vilket var en signifikant sänkning.

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