

CHANGES IN CALPROTECTIN CONCENTRATION IN SOW'S MILK THROUGHOUT LACTATION

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SUMMARY

Calprotectin is a protein of inflammation from neutrophils and activated macrophages. Faecal calprotectin is a non invasive marker of inflammation of the gastrointestinal tract in humans but little is known on its occurrence in milk. We determined kinetics of calprotectin in colostrums and milk throughout lactation in the porcine species. Milk was collected from fore and hind teats in nine sows between d0 and d29 of lactation (weaning at d28). Calprotectin was found in colostrums and milk of sows. Its concentration varied over time (peak at d5), position of the teat (fore < hind) and sow.

Key words: calprotectin, inflammation, lactation, milk, pig

INTRODUCTION

Calprotectin is a calcium-binding protein of 36.5 kDa that is released at sites of inflammation by neutrophil granulocytes and activated macrophages (Dale et al. 1985). This protein is resistant to degradation and displays anti-microbial properties *in vitro* (Dale et al. 1985; Brandtzaeg et al. 1995). In humans, faecal calprotectin is now considered as a valuable non invasive marker of inflammation of the gastrointestinal tract. Indeed, its concentration is elevated in inflammatory bowel diseases (review by Konikoff et al. 2006). Faecal calprotectin is also positively associated with lifestyle risk factors for colorectal cancer (Poullis et al. 2004). In the porcine species, calprotectin concentration in faeces was shown to be low in piglets at birth, and to increase in the first weeks of life (Lallès and Fagerhol, 2005). It was also 15-fold lower in specific pathogen-free as compared to conventional growing pigs (Lallès and Fagerhol, 2005).

The udder is a site of inflammation, especially around farrowing and after weaning in pigs. However, very little is known on the occurrence and role of calprotectin in this organ. In humans, the presence of calprotectin in milk has been occasionally reported but nothing more is known in connection with the cycle of lactation and on the possible transfer of calprotectin to the young via the milk.

As preliminary assays carried out in our laboratory indicated the presence of calprotectin in porcine milk (J.P. Lallès, unpublished), the aim of the present work was to investigate the changes in calprotectin concentration in colostrums and milk throughout lactation in sows.

MATERIALS AND METHODS

Animals and colostrums and milk sample collection

Nine pregnant Landrace x Large White sows from the experimental herd of INRA Saint-Gilles, France, were randomly selected for this study. All the sows were in good condition and none of them displayed any clinical sign of diseases. They were in the second to sixth parity and they nursed 11–14 piglets each. Colostrums were collected the day before (d-1) and the day (d0) of farrowing. Milk was collected at various times (d5, 7, 14, 21, 28) during lactation and once after weaning of the progeny (d29). Milk samples (15 to 30 ml) were obtained from two fore teats and two hind teats in the mornings (between 9.00 and 11.00) a few minutes after oxytocin administration intravenously in the ear (1.5 ml of oxytocin at 10 IU/ml). The two samples at each teat location were immediately pooled. The samples were frozen at -20°C until all the samples of the kinetics had been obtained. Then, the milk samples were thawed overnight at $+4^{\circ}\text{C}$. They were centrifuged at 4000 rpm for 15 min and the defatted colostrums or milk was collected using a glass Pasteur pipette.

Laboratory analyses

Calprotectin was extracted from defatted colostrums and milk in the extraction buffer (1:1, vol: vol; Calprest, Eurospital, Trieste, Italy) as used for faeces (Lallès and Fagerhol, 2005).

Calprotectin was assayed in these samples using a sandwich ELISA (Lallès and Fagerhol, 2005) following a protocol similar to that published for human calprotectin (Ton et al. 2000). In parallel, total protein was also determined in the defatted samples colorimetrically according to the procedure of Lowry et al. (1951).

Statistical analysis

Calprotectin data, contrary to total protein data were not distributed normally so they were \log_{10} -transformed before statistical treatment. The experimental unit was the sow. Data were analysed using the PROX MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) for testing the effects of time, teat position and sow and interaction between time and teat position. Significant effects were declared at $P < 0.05$. Data are presented as least-square means and SEM.

RESULTS

Total protein concentration over time in defatted colostrums and milk was influenced by the time ($P < 0.0001$), teat position ($P < 0.05$) and sow ($P < 0.05$) with no significant interaction between the time and teat position ($P > 0.05$). Total protein concentration decreased from 179 ± 6.4 g/L in colostrums the day of farrowing to 56 ± 6.4 g/L in the milk collected on d5 after farrowing without significant changes thereafter (mean concentration of 52 ± 2.5 between d7 and d29). Total protein concentration was 16% higher in the hind teats as compared to the front teats (79 ± 3.2 and 68 ± 3.3 g/L, respectively).

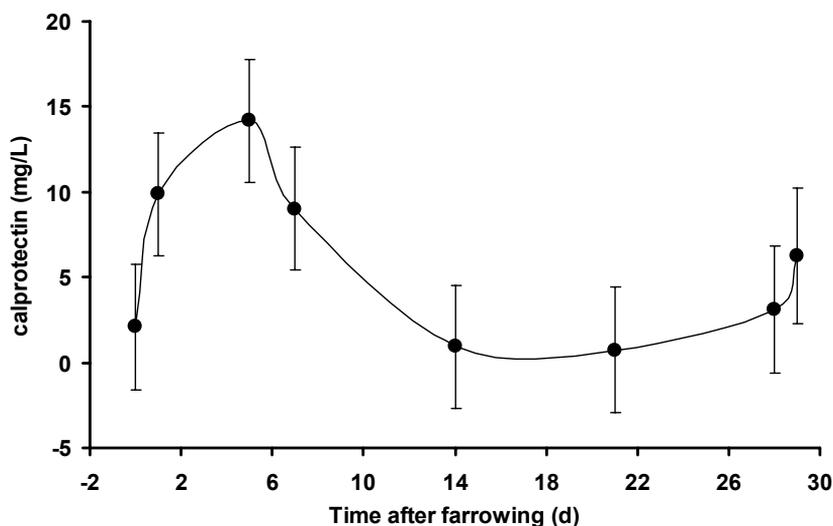


Figure 1. Changes in calprotectin concentration in defatted colostrums and milk of sows ($n = 9$) throughout lactation (weaning at d28) (Ismeans \pm SEM).

Calprotectin concentration in defatted colostrums and milk was very variable. It was influenced by the time, the teat position and the sow ($P < 0.0001$), the time by teat position interaction being non-significant ($P > 0.05$). Calprotectin concentration increased during the first days of lactation and then decreased progressively (Figure 1). Calprotectin concentration was 3.5-fold higher in the hind teats as compared to the fore teats (9.0 ± 1.8 and 2.6 ± 1.9 mg/L, respectively). This difference was more marked during the first week of lactation. Calprotectin concentration was influenced by the sow, two sows displaying values higher than 10 mg/L, two sows having values comprised between 5 and 10 mg/L and five sows showing much lower values (< 2.5 mg/L) (Figure 2).

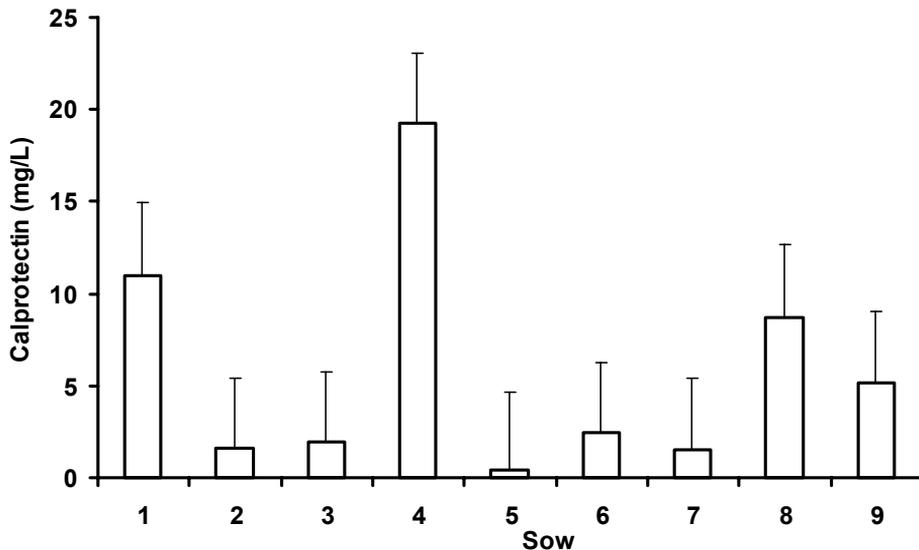


Figure 2. Influence of the sow on calprotectin concentration in defatted colostrums and milk (lsmeans \pm SEM).

DISCUSSION

It is known that oxytocin alters protein composition of milk in cows (Alex et al. 1985). Here we administered systematically low doses of oxytocin (15 IU per sow) as compared to higher doses (40 IU per sow) used in another study in sows (Elliot et al. 1984). This may have minimised the possible effect of oxytocin on protein composition of sow's colostrums and milk in the present work.

Total protein concentration and its observed decrease between colostrums and milk at the beginning of lactation are in agreement with previous data in the porcine species (review by Gallagher et al. 1997). However, this review emphasized that such a decrease does not occur uniformly for all protein types. This finding is supported further by the lack of correlation between total protein and calprotectin here.

The present results clearly show that calprotectin was present in colostrums and milk of sows throughout lactation. A low concentration of calprotectin was observed at farrowing suggesting a low level of mammary gland inflammation at that time. This observation is also in agreement with the low faecal calprotectin levels found in baby pigs at birth (Lallès and Fagerhol, 2005). The following increase in milk calprotectin level suggests an enhanced mammary gland inflammation during the first days of lactation. Whether it is stimulated by the process of suckling itself is presently unknown. As faecal calprotectin also increased during the first days of life in piglets, one question then arises on the origin – maternal via the milk or endogenous – of faecal calprotectin in piglets.

The kinetics of calprotectin concentration in colostrums and milk as revealed here appeared to differ substantially from that of lactoferrin. Lactoferrin is a multifunctional protein with anti-inflammatory and bacteriostatic properties, found in milk, and originating from glandular

epithelial cells and neutrophil granulocytes (review by Ward et al. 2005). Lactoferrin concentration was found to be high in sow's milk during the first week of lactation in two studies (Elliot et al. 1984; Yang et al. 2000). Ceruloplasmin, an acute-phase protein enhanced by inflammation and inflammatory cytokines is also higher in early than in late lactation in sows (Cerveza et al. 2000). Despite distinct kinetics, all these proteins display higher levels in early lactation, indicating this period is critical for inflammation.

Another interesting point of this study is the influence of teat position on calprotectin concentrations. The hind teats displayed much higher calprotectin levels than the fore teats, especially during the first week of lactation. One hypothesis is that hind teats would be more exposed to bacterial contamination by sow's faeces. It is known that improving hygiene of the farrowing crate by removing faeces decreased bacterial contamination of the mammary gland and mastitis in sows (Bertschiner et al. 1990). However, to the best of our knowledge no published information is available on a possible link between teat position and inflammation.

CONCLUSIONS AND IMPLICATIONS

In conclusion, calprotectin was found in the colostrums and milk of sows. Its concentration varied over lactation independently from total protein concentration. Calprotectin concentration in milk depended on the position of the teat, hind teats displaying much higher levels than fore teats. Calprotectin may be a valuable biomarker of mammary gland and teat inflammation. Calprotectin in colostrums and milk might be protective not only to sow's udder but also to the gastrointestinal tract of the progeny.

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