

FAECAL CALPROTECTIN: A NON INVASIVE MARKER OF INFLAMMATION IN PIGS?

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Introduction

In farm animals, non-invasive markers for evaluating the degree of gut inflammation according to factors dealing with diseases, hygiene, environment and nutrition are lacking. By contrast, in humans, faecal calprotectin is considered as such a marker due to its high resistance to digestion by host enzymes and degradation by gut microbes. Increased concentrations in faeces have been demonstrated in various inflammatory diseases, including necrotic enterocolitis in preterm infants (Carroll et al., 2003) and inflammatory bowel diseases (ulcerative colitis and Crohn's disease) in infants (Bunn et al., 2001) and adults (Costa et al., 2003). Recently, it was shown that faecal calprotectin was associated with lifestyle risk factors for colorectal cancer (Poullis et al., 2004). Calprotectin is a calcium-binding trimeric protein of 36.5 kDa, released at sites of inflammation by neutrophil granulocytes and activated macrophages, and showing anti-microbial properties *in vitro* (Dale et al., 1985; Brandtzaeg et al., 1995). Calprotectin is also found in human milk, urine, cerebrospinal fluid, and keratinocytes of mucous membranes and in skin during skin diseases. An enzyme-linked immunosorbent assay (ELISA) is commercially available. However, since this kit is specific for human calprotectin, reagents and an ELISA assay were developed for pig calprotectin (M.K. Fagerhol, unpublished data) and served for determining reference values in pigs in the present study.

Materials and Methods

A sandwich ELISA assay specific for porcine calprotectin was set up and used with purified porcine calprotectin as the standard (M.K. Fagerhol, unpublished data). This ELISA was used for determining reference values in pigs of various ages and sanitary status (for details see Table 1). Faeces samples were collected fresh and stored frozen at -20°C until analysis. Calprotectin was extracted and assayed essentially as described for humans (Ton et al., 2000). Since preliminary data suggested faecal calprotectin to be lower in sow-fed piglets as compared to babies (M.K. Fagerhol and J.P. Lallès, unpublished data), various extraction conditions were studied (100 mg fresh faeces in 5, 2.5 or 1.25 ml of extraction buffer). The

data are expressed in mg per kg fresh faeces. Values above 100 mg/kg were considered as high and the corresponding pigs were excluded from the results presented here.

Results

Calprotectin level was found to decrease as the concentration of faeces in the extraction buffer increased (Figure 1). Therefore, the ratio recommended for humans (100 mg faeces per 5 ml buffer) was used throughout this study. However, only 25 mg faeces were used to save extraction buffer since this did not appear to influence calprotectin concentration (data not shown).

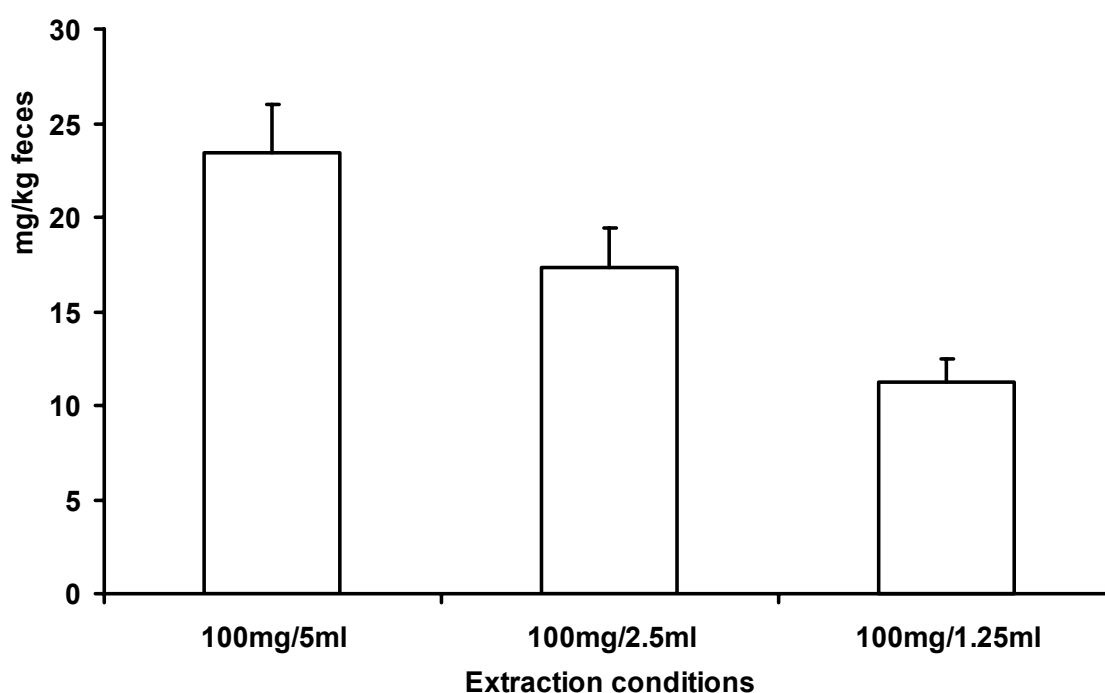


Figure 1. Influence of the fresh faeces to extraction buffer ratio on calprotectin concentration in pig faeces (means and SEM, n = 9 per treatment)

Sows peri-partum (7 d before to 7 d after farrowing) had mean and upper reference (+ 2SD) values of 13 (38) mg/kg faeces (Table 1). The values were 24 (60) and 33 (85) mg/kg in unweaned piglets at d 0 and d 1-4 of age, respectively, for samples with values below 100 mg/kg. However, in the whole studied population, the percentage of piglets with at least one value superior to 100 mg/kg faeces increased soon after birth (4, 24 and 46% of the piglets at d 0, d 1 and d 4, respectively). Unweaned piglets of 10-20 and 28 d of age had faecal concentrations of calprotectin of 30 (86), and 21 (58) mg/kg. Conventional growing pigs of 40 and 80 kg BW had values of 38 (78) and 30 (72) mg/kg, respectively. By contrast, SPF pigs

of 80 kg BW had a value of 2 (5) mg/kg faeces, that is 15-fold less than conventional growing pigs of the same BW.

Table 1. Calprotectin concentrations in the faeces of various categories of pigs

Category	Calprotectin concentration (mg/kg faeces)					
	Mean	SD	n	Min	Max*	Mean + 2SD
Sows peri-partum (-7 to + 7 d)	13	12.5	61	1.2	71	38
Piglets, d 0 (birth)	24	18	25	3.6	80	60
Piglets, d 1-4	33	26	34	0.8	95	85
Piglets, 10-20 d, unweaned	30	28	12	2	84	86
Piglets, 28 d, unweaned	21	18.5	35	4	90	58
Growing pigs, 40 kg BW	38	20	55	10	82	78
Growing pigs, 80 kg BW	30	21	10	10	86	72
Growing SPF pigs, 80 kg BW	1.9	1.6	41	0.6	9.5	5.1

* values higher than 100 mg/kg not considered in this table

Discussion

To our knowledge, these are the first data on concentrations of calprotectin in faeces in the porcine species. The conditions of calprotectin extraction appear to be critical, as already outlined for human samples (Ton et al., 2000). In humans, median values of 12 (range 2-47) mg/kg have been reported for healthy adults (Bunn et al., 2001). By contrast, these values were shown to be high in newborns (means and SD of 145 ± 78.5 mg/kg, Laforgia et al., 2003). They were also high in infants aged 6 weeks (562 ± 610 mg/kg) but they decreased in time up to five years of age (81 ± 47 mg/kg) (Rugtveit and Fagerhol, 2002). Here, values for adult pigs (sows) appeared to be close to data reported for adult humans. Values in piglets at birth did not appear to be as high as in babies. However, we observed increased levels of faecal calprotectin in piglets between d 0 and d 4 after birth. No clinical signs of diseases in the studied litters were recorded and, therefore, we have no precise explanation for these high values at the moment, Mean values were between 20 and 40 mg/kg in older, unweaned piglets and in growing pigs. Finally, the data obtained in SPF pigs clearly indicate that faecal calprotectin (and most probably gut inflammation) is very low in these pigs with a high sanitary status.

Conclusion

Mean concentrations of calprotectin in the faeces of adult pigs appeared to be in the same range as in healthy adult humans. They were much lower at birth in piglets than in babies. They increased soon after birth in piglets and were influenced by the sanitary status in growing pigs. The present data show the potential of faecal calprotectin as a non-invasive marker of gut inflammation (and sanitary status?) in the porcine species. Further work is needed to extend these observations, to investigate changes in young piglets in more detail and finally to ascertain whether weaning and rearing conditions influence the faecal levels of this marker.

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