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BRIEF COMMUNICATION: Are serum amyloid protein levels in milk an indicator of mastitis infection in New Zealand dairy goats?

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INTRODUCTION

The incidence of clinical mastitis in dairy goats is low at less than 5% per lactation (Bergonier *et al.*, 2003), however, there is a recognised need in the industry for a simple, practical and reliable means for the detection of sub-clinical mastitis. In goats, conversely to cows, somatic cell counts (SCC) are not a reliable indicator of infection. Bacteriological cultures can be used to identify glands with sub-clinical infection, but this approach involves specialized sampling. Serum amyloid A (SAA) is a family of acute phase proteins that is produced in response to infection and inflammation. Levels of this protein in milk have been proposed as a sensitive indicator of mastitis infection in the dairy cow (Akerstedt *et al.*, 2007; Jacobsen *et al.*, 2005; O'Mahony *et al.*, 2006; Weber *et al.*, 2006) and in dairy sheep (Winter *et al.*, 2005). Conversely, one report in the literature suggests that this relationship does not hold true in dairy goats (Winter *et al.*, 2005).

The SAA is a 12.8 kDa protein that has several different isotopes. It is induced primarily in the liver by the pro-inflammatory cytokines IL-1, IL-6 and TNF- α (Yamada, 1999). A mammary isotype of the protein has also been described in the milk of several different species, including the cow, horse and sheep. This form of the protein, produced by mammary epithelial cells, is abundant in colostrum. However, in milk from healthy animals the levels are low (Jacobsen *et al.*, 2005; McDonald *et al.*, 2001). It has been suggested that SAA in mammary secretions may have an important protective role in the gastrointestinal tract of the neonate and/or the healthy maintenance of the mammary gland (Eckersall *et al.*, 2006; McDonald *et al.*, 2001).

The aim of this study was to examine the relationship of SAA, SCC and bacterial infection in milk from dairy goats and determine whether levels of SAA could be used for the detection of sub-clinical mastitis in this species.

MATERIALS AND METHODS

Milk samples

Foremilk samples were collected following aseptic teat-end preparation from a random selection of dairy goats in early and again in mid-lactation.

Two time points were assessed to check for differences that may occur due to stage of lactation. The milk samples were assessed for bacterial presence following the National Mastitis Council guidelines. Milk samples from which >2 CFU/10 μ L of one or two pathogens were identified were defined as having an intra-mammary infection. The SCC was determined by standard methods (Fossomatic, Foss, Denmark). Samples of whole milk were stored at -20°C.

A sub-group of 200 milk samples was chosen for SAA analysis by randomly selecting 50 samples within each time point and within each infection category of growth or no growth.

Serum amyloid assay

The concentration of SAA was determined in selected milk samples using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Tridelta Mast ID range Milk Amyloid A assay, Tridelta Development Ltd, Kildare, Ireland), first described by McDonald *et al.* (1991). This assay, which measures both hepatically-derived and mammary-derived forms of SAA from a number of different species, was undertaken according to the manufacturer's recommended protocol. Briefly, standards or samples plus biotinylated monoclonal SAA antibody were incubated in microtitre plate wells pre-coated with capture monoclonal SAA antibody. In one step, SAA in the standard or sample, was captured and labelled in a sandwich format. After washing to remove all of the unbound material, wells were incubated with streptavidin-horse radish peroxidase prior to the addition of enzyme substrate (3,3',5,5'-tetramethylbenzidine, TMB). The reaction was stopped with the addition of 2 M sulphuric acid. Optical density in the wells was measured at 450 nm using an automated plate reader (Versa_{max}; Molecular Devices, CA, USA). A standard curve was constructed by plotting SAA concentration versus optical density for determining the unknown SAA concentrations of samples. Samples were initially diluted to 1:500 for assay. Samples with an optical density outside the range of the standard curve were diluted further and re-analysed.

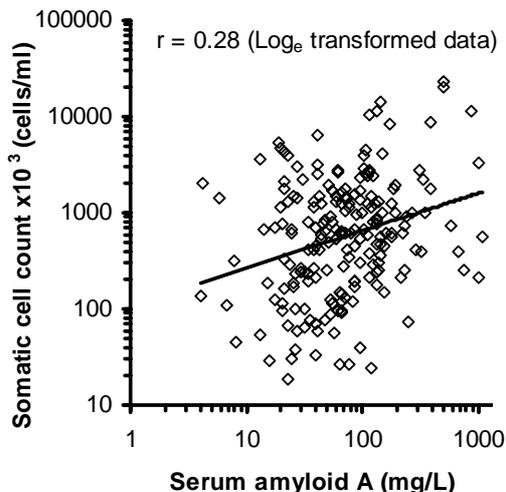
For data analysis, the SAA concentrations and SCC were transformed to log_e due to non-normal

distributions. Analysis of variance was performed with the SAA concentration as the dependent variable and with the presence or absence of bacterial growth and early or mid stage of lactation as the independent variables.

RESULTS AND DISCUSSION

In goat milk samples with no detectable bacteria, the geometric mean of SAA was 61.5 mg/L with values ranging from 4.0 to 1,102.3 mg/L. In goat milk samples with bacterial growth the SAA level was higher at 76.7 mg/L. The difference was not significant ($P = 0.325$). In goat milk samples with bacterial growth, the geometric mean SCC was 804,000 cells/mL compared with 404,000 cells/mL for samples with no growth ($P < 0.001$). The correlation between SCC and SAA levels ($r = 0.28$, $P < 0.001$) suggested that there was only a small relationship between the level of SAA and SCC in goat milk. Figure 1 shows a comparison between these two parameters.

FIGURE 1: A comparison of the somatic cell count and serum amyloid A levels measured in selected milk samples collected from goats in early and mid-lactation.



In goat milk samples with no growth, there was no significant difference ($P = 0.06$) in the geometric mean SAA determined in the early and mid season of 51.6 and 73.2 mg/L respectively. However, over this same time period the geometric mean SCC for these milk samples increased from 185,000 to 870,000 cells/mL ($P < 0.001$). These data suggest that the level of SAA in goat milk does not have a strong relationship with the SCC in the milk. This is in contrast to the reported findings in the cow (Akerstedt *et al.*, 2007; O'Mahony *et al.*, 2006) and the sheep (Winter *et al.*, 2006), where a significant relationship was shown between SAA and SCC.

Overall, these data do not support the use of SAA levels in milk samples for detection of sub-clinical mastitis infection in dairy goats; as has been

previously reported by Winter *et al.* (2005). Levels of SAA between individual animals varied widely and were independent of the presence of bacterial growth. SAA may not play the same role as an acute phase response protein in the goat mammary gland as in other species.

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