Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

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Abstract

At present mastitis in dairy cattle is detected by using somatic cell count and various bacterial diagnostic tests. Still it is difficult to understand the exact health status of the animal by mere observation of the test results, since there maybe presence of sub-clinical cases. There are indications in other species like poultry that natural antibodies might be used as indicators for the identification of (more) disease resistant individuals (Wijga, 2009). The present study focuses on the relationship between the natural antibody titres collected at a single sampling moment and the somatic cell count later inn the lactation. If proven, natural antibodies can be used as indicators for the immune status of the animal. For the present research the preliminary analysis of the fixed effects were analysed using the SAS software and the genetic parameters were estimated using the ASReml software. The results from the analyses show that relationships between the NAb titres and SCS after the sampling moment exists, but with a trend expressing a positive correlated response. The study shows that the NAb levels do not show a protective effect on the animal as the cell counts later in the lactation increased when the NAb levels increased. Hence NAb titre measured in milk samples may not be useful as a tool against mastitis selection

Keywords: Mastitis, somatic cell score, Natural antibodies, relationship, heritability.

Introduction

Mastitis is an economically important disease in dairy cattle caused mainly by bacteria, but also by viral or fungal pathogens, leading to inflammation and subsequent pathological changes in the udder. It still remains a major cause for economic loss in the dairy industry. A much desired preventive measure against mastitis is selection of mastitis resistant animals. Improving the immune status of the animal is one way in this direction. Until now most research has focussed on selection based on somatic cell count of milk. (Miller and Paape, 1985, Kehrli and Shuster, 1994, Smith, 1996).The somatic cell count (SCC) in milk is affected by the infection status of the mammary gland and hence reflects the health condition of the animal (Miller and Paape, 1985). These cells include lymphocytes, macrophages, polymorphonuclear (PMN) cells and some epithelial cells (Pillai, et al, 2001),

which shows elevated levels during infectious condition, indicating an active immune response. During infection the number of inflammatory cells increase considerably, for example, PMN cells increase from 5-12 percent to 90 percent (Kehrli and Shuster, 1994). The genetic correlation between lactation mean SCC and clinical mastitis (CM) is observed to be positive (Philipsson et al., 1995) and ranges from 0.3 (Weller et al., 1992) to 0.79 (Philipsson et al., 1995). Smith (1996) proposed that cows with somatic cell count more than 300,000 cells/ml are probably clinically infected.

Natural antibodies (NAbs) are a part of innate humoral defence mechanism. The four bovine natural antibodies are the immunoglobulins – IgA, IgG_1, IgG_2, and IgM. NAbs provide an initial broad protection against micro-organisms (Rainard & Riollet, 2006). NAbs have a regulatory function in initiating immune system (Ochsenbein et al., 1999) and react to initial infection by detection of certain pathogen-associated molecular patterns (PAMPs) (Bannerman et al., 2004). It was demonstrated by van Knegsel et al. (2007), that NAbs can be determined in milk. It is hypothesized that animals with high NAbs have an increased immunity and thus less susceptible to mastitis (Grabar, 1983, Tomer and Shoenfeld Y., 1988, Ochsenbein et. al., 1999).

de Weerd (2009) showed that IgM levels in milk was negatively correlated with the probability of incidence of mastitis within 90 days after the sampling moment. This is an indication that a cow low in IgM, is more susceptible to infection. de Weerd also observed that the IgG1 level was positively correlated to SCC later in lactation, suggesting that IgG1 can be used as early predictor for the probability of infection. This indicates that a cow having a high IgG1 titre would have a high SCC, indicating presence of infection. The IgM level was found to be predictive for resistance against mastitis within 90 days after sampling. In the above study, family relations that existed in the data were not accounted for. Wijga (2008) studied the relation between somatic cell count and NAbs measured in the same milk sample. Wijga (2008) showed moderate genetic correlations of –0.48 between the antigen peptidoglycan (PGN) and SCS (somatic cell scores, which are the natural logarithms of SCC) to a positive genetic correlation of 0.35 between SCS and the antigen lipoteichoic acid (LTA). The results from by de Weerd (2009) and Wijga (2008) are promising, and more exploration can be done by incorporating the pedigree information which was not used in the studies. So the results may help to identify and utilize more reliable predictors indicating the immune status of the animal. The present study utilizes the same dataset used by de Weerd

and Wijga, where the present work concentrates more on the relationship between the NAb titres at a sampling moment and the SCS later stages of lactation.

The aim of the present study is to estimate the relationship between the natural antibody level collected at one sampling moment and the somatic cell count later in that lactation.

Materials and methods

The total dataset consisted of 2025 cows in their first parity originating from 398 farms. These animals were part of the Milk Genomics project that emphasizes on the genetics of milk composition. The farms took part in the Milk Production Registration (MPR) which is a system for recording milk production and udder health status of the cows. Milk samples are collected at regular intervals of 3, 4 or 6 weeks and analyzed for somatic cell count, fat and protein percentage. Information available on milk samples are NAb titres at a single sampling moment, the routine SCC measurements, dates of sample collection, calving season, the calving dates, the calving age and days in lactation.

Milk samples collected from the cows are analyzed at regular intervals of 3, 4 or 6 weeks for SCC. The somatic cell count is converted to their respective somatic cell score (SCS) by using the natural logarithm values. An aliquot of the milk sample was taken during the regular milk recording and used to measure NAbs binding to specific pathogen associated molecular patterns (PAMP). NAb binding was determined by using an indirect ELISA technique (van Knegsel et al., 2007). The NAbs binding to keyhole limpet hemocyanin (KLH), lipopolysaccharide (LPS) from *E. coli*, lipoteichoic acid (LTA) from *S. aureus* and peptidoglycan (PGN) from *S. aureus* were determined in individual samples. The moment at which the sample is taken for the analysis of the NAb levels is referred to as the "sampling moment." These observations are used to determine the relationship between the natural antibody titres measured at one test-day and the somatic cell counts later in that lactation. It is also analysed whether the cell count at the sampling moment had significant effects on the before and after cell counts in the lactation and compared with the effect of the NAb titres on the cell counts after the sampling moment.

The LTA antibodies are classified into different immunoglobulin isotypes as LTAG1 for IgG1, LTAG2 for IgG2, LTAA for IgA and LTAM for IgM. There is also a measurement of IgG1, IgG2, IgA and IgM together denoted as LTA. For the LPS, PGN and KLH, the

measurement of the total antibodies are observed and represented as LPS, PGN and KLH respectively.

Cell count traits

Cell count traits analysed include include the average SCS before (**b4scs**), SCS at (**SCSsample**), the average SCS after the sampling moments (**afscs**), the standard deviation of the SCS before (**b4stdscs**) and the standard deviation of SCS after the sampling moment (**afstdscs**). Traits are defined as:

SCS at the sampling moment:

$$\text{SCSsample} = \text{Ln}(\text{scc}/1000)$$

SCS before the sampling moment:

$$\text{b4scs} = \sum_{i=1}^{n} (\text{Lnb4scc})/n$$

SCS after the sampling moment:

$$\text{afscs} = \sum_{i=1}^{n} (\text{Lnafscc})/n$$

Standard deviation of the SCS before the sampling moment:

$$\text{b4stdscs} = \sqrt{1/(n-1)\sum_{i=1}^{n} (\text{b4scs} - \overline{\text{b4scs}})^2}$$

Standard deviation of the SCS after the sampling moment

$$\text{afstdscs} = \sqrt{1/(n-1)\sum_{i=1}^{n} (\text{afscs} - \overline{\text{afscs}})^2}$$

where n corresponds to the number of test days contributing to the mean or standard deviation.

Data editing

The number of days in lactation beyond 400 days is eliminated in the lactation days after the sampling moment. The number of samples per animal before and after the sampling moments is also calculated. The mean values of the somatic cell score before, at and after the sampling moment are calculated with the minimum value per animal being more than 1. The standard deviations for SCS before (**b4stdscs**) and after (**afstdscs**) sampling moment were calculated and the minimum number of samples per animal was taken as 4.

Statistical analysis

The general linear model (SAS, PROC GLM procedure) is used for statistical analysis. The statistical analysis to estimate the relationship between natural antibody titres

and the somatic cell count later during the lactation are performed in ASREML. Depending upon the trait analysed, slightly different models were used. The base model is:

$$\mathbf{y_{ijkl} = \mu + b_1 NAb_{ijkl} + b_2 (NAb)^2_{ijkl} + b_3 lacst_{ijkl} + b_4 e^{-0.05 lacst}_{ijkl} + b_5 ca_{ijkl} + b_6 ca^2_{ijkl} + season_i + sirecode_j + herd_k + U_l + e_{ijkl}} \quad (1)$$

$\mathbf{y_{ijkl}}$- dependent variable corresponding **μ** – overall mean. **lacst**- the lactation stage in days. $\mathbf{e^{-0.05lacst}}$- variable which explains the lactation in days modelled with a Wilmink curve (Wilmink, 1987) **ca**- the calving age in days, $\mathbf{ca^2}$-the squared value of **ca**. **NAb** is the logarithm base 2 values of natural antibody titre for either LTAG1, LTAG2, LTAA, LTAM, LTA, LPS, PGN or KLH. **b**- regression coefficient for the respective variables. A linear and a quadratic component was modelled. **herd**, represents random effect of the herd with a distribution, herd ~ $N(0, I\sigma^2_{herd})$. **sirecode** is the fixed sire effect taking possible differences between daughters of proven bull and the test bulls into account. **season** is the fixed effect for the season of calving, which includes three classes: summer, autumn and winter (June to August 2004, September to November 2004 and December 2004 to February 2005 respectively). $\mathbf{U_r}$, random additive genetic effects of animal **r** with U ~ $N(0, A\sigma^2_U)$, $e_{ijklmnopq}$, random residual effect with e ~ $N(0, I\sigma^2_e/wt)$, where wt- number of observations that contributed for the mean and standard deviations of the SCS before and after; for the SCS at the sampling moment no weighted analysis was used. The random residual effect for the SCS at the sampling moment was e ~ $N(0, I\sigma^2_e)$.

Description of the model

Linear and quadratic relationships between each NAb and the SCS at different periods are estimated one at a time. For the **SCSsample**, there was a single observation for each animal and the **lacst** and $\mathbf{e^{-0.05lacst}}$ were used in the model. For the **afscs** and **afstdscs,** the **aftlacst** and **afCOUNT** were used for the model instead of the **lacst** and $\mathbf{e^{-0.05lacst}}$. For the **b4scs** and **b4stdscs**, the **b4lacst** and the **b4COUNT** were used in the model. The corresponding days in lactation and the number of samples were used for the mean and standard deviations of SCS before and after. The season, sire and herd are the class variables and the number of samples taken per animal after the sampling moment (**afCOUNT)** and before the sampling moment (**b4COUNT)** are used for weighted analysis for the ASReml estimations.

Model (1) without the effect of the NAb titres as co-variable was used to estimate heritabilities for the different cell count traits. Here, again the same basic model without the NAb titres was used for the different cell count traits. For the mean cell count traits before and the after the sampling moment, the lactation stage in days and the number of test days per animal was considered accordingly. For the standard deviation of the cell counts before and after, their corresponding lactation stage in days and the corresponding number of test days taken to find the standard deviations were accounted in the above model as well. For the cell counts at the sampling moment, the lactation in days for that moment was changed in the model accordingly without the number of the test days. Heritabilities (h^2) were estimated using a univariate analyses and were calculated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_e^2} \qquad (2)$$

where σ_A^2 is the additive genetic variance and σ_e^2 is the residual variance.

Results

The exploratory analysis were performed using the general linear model. The fixed effects were corrected using SAS software and the statistical genetic analysis were run using the ASReml software. The estimates obtained from the analysis from SAS were used to find the phenotypic correlations and also to find out the relation between the NAbs at the sampling moment and the cell counts taken after the sampling moment later in the lactation. The table 1 shows the number of animals, mean and the standard deviations observed for the different cell count traits.

Table 1. number of animals, mean and standard deviations for the mean SCS before, at and after and also for the standard deviation of the SCS before and after sampling moment.

	No. of animals	Mean	Standard deviation
b4scs*	1900	4.02	0.82
SCSsample	1939	3.77	1.09
afscs	2025	4.34	0.87
b4stdscs	1893	0.62	0.42
afstdscs	1921	0.46	0.34

b4scs* = the mean somatic cell score before the sampling moment

SCSsample = somatic cell score at the sampling moment

afscs = the mean somatic cell score after the sampling moment

b4stdscs = Standard deviation of the somatic cell score before the sampling moment

afstdscs = Standard deviation of the somatic cell score after the sampling moment

Relations of NAb titres with mean SCS before, at and after

The analysis show that, somatic cell scores of the cows taken before, at and after the sampling moment showed significant relations with the antibody titres (Table 2). The sqLTAG1 ($p<0.0001$), LTAG2, sqLTAG2, sqLTAM, sqLTAA, sqLTA, KLH and sqPGN all showed significant relationships on the SCS before. The lactation in days and the NAb tires of LTAG2 ($p<0.0001$), sqLTAG2 ($p<0.0001$), sqLTAM, sqLTAA and LTA had significant effects on the SCS recorded at the sampling moment. For the SCS after the sampling moment the significant effects were observed for the lactation in days ($p<0.0001$), sqLTAG1, sqLTAG2, sqLTAM, sqLTAA and the sqLTA. It was also analysed whether the SCS at the sampling point had significant effects on the before and after SCS and was found to have significant relations.

Relations of NAb titres with standard deviation of SCS before and after

The significance of effects for the standard deviations of the SCS before sampling moment are shown (table 2) by days in lactation ($p<0.0001$) and sqLTAG2, PGN and sqPGN. Significant effects for standard deviation of SCS after the sampling moment were observed for days in lactation and calving season. For standard deviation of SCS before and after sampling moment, the days in lactation showed very significant effects. The effect of lactation days in the SCS before the sampling moment was observed to be negative, whereas the estimated effect of the lactation in days for the SCS after the sampling moment was observed to be positive (estimates not shown).

Table 2. The significance of the effect of lactation stage, season of calving and natural antibodies on the traits mean and standard deviation of SCS before, at and after the sampling moment.

	[b]Lactation stage	season	[c]LTAG1		LTAG2		LTAM		LTAA		LTA		KLH		LPS		PGN		SCSsample	
			L[d]	Q	L	Q	L	Q	L	Q	L	Q	L	Q	L	Q	L	Q	L	Q
[a]b4scs				***	**	*		*		**		**	*					**	***	
SCS-sample	*				***	***		**		**	**								-	-
afscs	***			**		*		*		**		**							***	*
b4stdscs	***					**											**	**	***	***
afstdscs	**	**							*										***	***

[a]b4scs- SCS before sampling moment, SCSsample- SCS at sampling moment, afscs- SCS after sampling moment, b4stdscs- standard deviation of b4scs, afstdscs- standard deviations of afscs.

[b]lactation stage - days in lactation for the before, at and after sampling moments.

[c]LTAG1, LTAG2, LTAM, LTAA, LTA, KLH, LPS and PGN- linear and quadratic expressions of NAb titres binding to respective molecular antigens. significant effects p <0.05=*, <0.005=**. <0.0001=***

L[d] – Linear (L) and quadratic (Q) components of the NAB titires at the sampling moment

Table 3. The significant relations of the linear NAb titres the mean and standard deviation of SCS before, at and after the sampling moment. significant effects p <0.05=*, <0.005=**. <0.0001=***

	LTAG1	LTAG2	LTAM	LTAA	LTA	KLH	LPS	PGN	SCSsample
b4scs*	***	**	***	***	***	***	***	***	***
SCSsample	***	***	***	***	***	***	***	***	-
afscs	***	***	***	***	***	***	***	***	***
b4stdscs	**		***	**	**	*	**	***	***
afstdscs					*	***		*	*

b4scs*= the mean somatic cell score before the sampling moment

SCSsample = somatic cell score at the sampling moment

afscs = the mean somatic cell score after the sampling moment

b4stdscs = Standard deviation of the somatic cell score before the sampling moment

afstdscs = Standard deviation of the somatic cell score after the sampling moment

Relations of the linear effect of NAb titres on the mean and standard deviation of SCS before, at and after the sampling moment

From the results shown in table 2, it was observed that not all of the quadratic expressions of the NAbs for the SCS at different sampling moment did not show significant relationships. Hence the same model was run using only the linear expression of the NAb titres against the different SCS to analyse the relation of the linear component of NAb titres with the different cell count traits. The results are shown in Table 3. It is observed that the linear expression of the NAb titres for LTA, KLH, LPS and PGN showed more significant relations to the cell count traits compared to the quadratic expressions of these NAb titres. So, for all the NAb titres where the quadratic component was significant, it shows that the quadratic component of the NAb titres explains more of the cell count traits. If both the quadratic and the linear components of the NAb titres were not significant, then neither of the two components showed any significant relations with the cell count traits. And, if only the linear component was observed to have significant relation with cell count traits, then the quadratic component could be avoided.

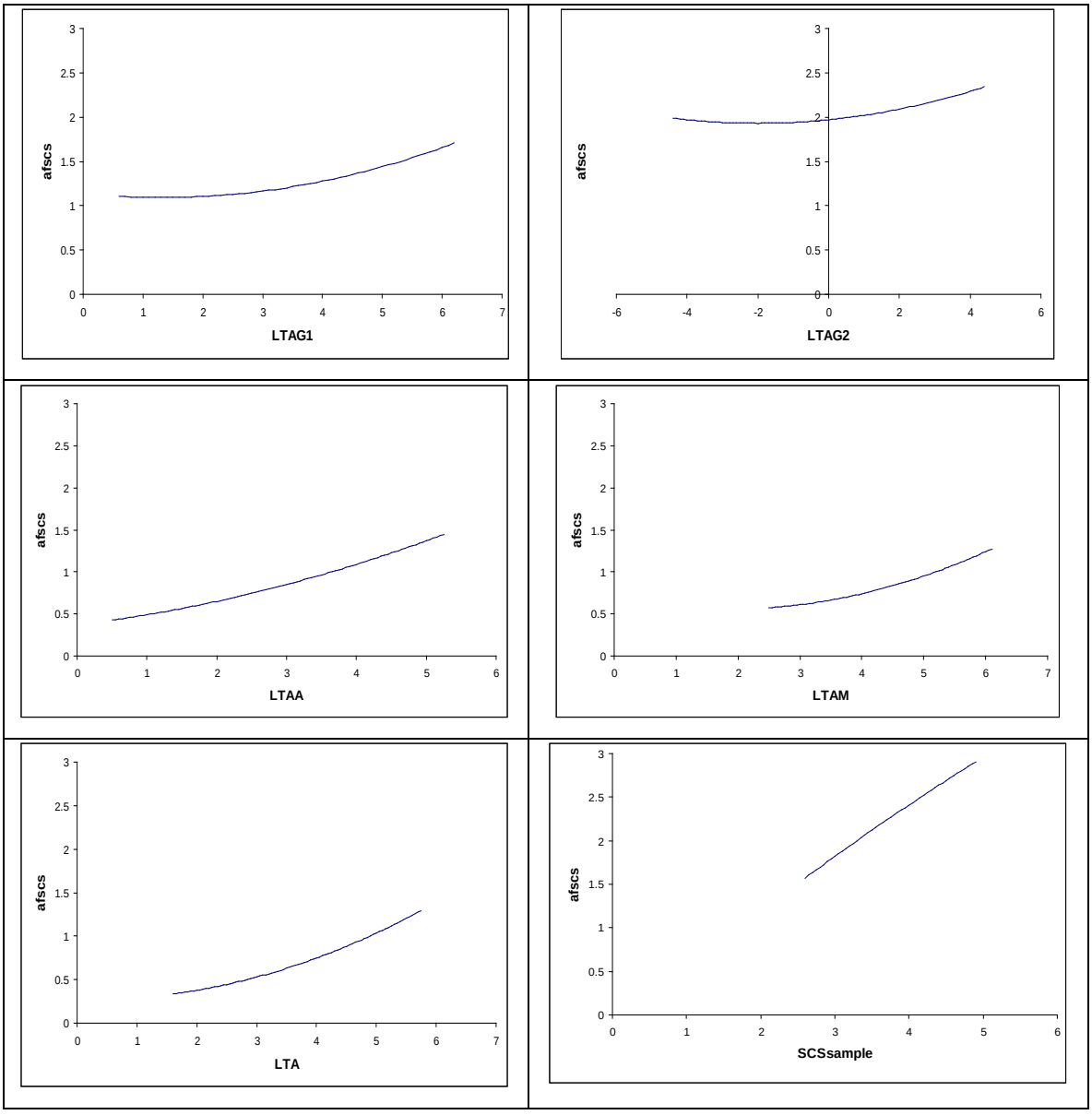
In short it was observed that for the cell counts before, the quadratic component of LTAG2, LTAA, LTAM and the PGN and the linear component of KLH and LPS showed significant relations. For the cell counts at the sampling moment, the quadratic components for LTAG1, LTAG2, LTAA, LTAM, and LTA and the linear components of KLH, LPS and

PGN showed significant relations. The quadratic expression of the NAb titres for LTAG1, LTAG2, LTAA, LTAM, LTA and the linear expression for KLH, LPS and PGN were significant related to the cell counts after the sampling moment. For the standard de3viation of the cell counts before the sampling moment, the quadratic component for LTAG2 and PGN and the linear components for LTAA, LTAM, LTA, KLH and LPS were found to be significantly related. Only the linear components of LTA, KLH and PGN showed significant relations with the standard deviations of the cell counts after the sampling moment.

Significant relationship between the Natural antibodies at the sampling moment and the SCS after the sampling moment

The estimated relationship between the significant NAb titres collected at the sampling moment and the SCS after the sampling moment are shown in figure 1. Only those variables which showed significant effects on the SCS after the sampling moment are shown.

Figure 1. The relationship between the NAb titres at the sampling moment and the SCS after (afscs) the sampling moment is shown below.



The curves depicting the relationship between the NAb titres and the SCS after the sampling moment which showed significant effects on the SCS recorded after the sampling moment, all show a trend for the cell counts later in the lactation in such a way that the SCS after the sampling moment increase with increase in NAb titres.

Heritability

Natural antibody titres and SCS at the sampling moment

The heritability for the antibodies and the SCS at different sampling moment ranges from 0.53 for LTAA to 0.01 for b4stdscs before the sampling moment. Among the NAb titres

the highest heritability was observed for LTAA NAb titres (0.53)and the lowest for LTAG2. Among the different SCS heritability estimates the highest was observed for afscs (0.30) and lowest for the b4stdscs (0.01)

Table 3. Phenotypic variance (σ^2_P) and the heritability (h^2) for the SCS at the sampling moment and the NAbs LTAG1, LTAG2, LTAM, LTAA, KLH, LPS, LTA and PGN

	σ^2_P	h^2
LTAG1	1.40 (0.05)	0.10 (0.06)
LTAG2	3.32 (0.13)	0.09 (0.05)
LTAM	0.70 (0.03)	0.47 (0.09)
LTAA	1.07 (0.05)	0.53 (0.11)
KLH	0.49 (0.02)	0.42 (0.09)
LPS	0.83 (0.03)	0.16 (0.06)
LTA	0.72 (0.03)	0.32 (0.08)
PGN	0.80 (0.03)	0.13 (0.06)
SCS	1.05 (0.04)	0.08 (0.05)
afstdscs	0.32 (0.03)	0.16 (0.04)
afscs	1.65 (0.14)	0.30 (0.06)
b4scs	2.45 (0.20)	0.08 (0.03)
b4stdscs	0.86 (0.04)	0.01 (0.01)

[1]standard errors are in parentheses

Discussion

Natural antibody titres and SCS

In the present study it was observed that the natural antibodies showed significant relations with the SCS before, at and after the sampling moment. It is evident from the estimates (not shown) and from figure 1, that the NAbs and the SCS share a relation which is positive. The studies by Knegsel et. al. (2009) confirmed that NAbs showed a relation to the SCC, which indicates trends for relations between increased NAb concentrations and mammary infections. The KLH antigen is from the sea mollusc and is usually not encountered in the environment of the dairy cattle. Therefore, KLH is a totally new antigen to the animal. Antibodies to KLH therefore are expected to give an idea about the level of NAbs present in the animal. The LTA, PGN and LPS are from bacteria and unlike the KLH antigen, these antigens are normally present in the environment, so the antibody titres against these antigens give an idea of the active status of the immune system (van Knegsel et al., 2007).

Natural antibody titres and the SCS later in lactation

The focus of the study was to observe the relation of the NAb titres collected at the sampling moment and the somatic cell score later in the lactation. This was compared to the relation between the NAb titres with the SCS before and the SCS at the sampling moment. The tables 2 and 3 show that there was not much difference in the significant relationships between the NAb titres and the different cell count traits. There was difference in the heritabilities for the different cell count traits where the cell count traits after the sampling moment was higher (0.30) than the cell count traits at and before the sampling moment (0.08 for both).

Estimates show that when NAb titres increase, the SCS after the sampling moment also tends to increase (figure 1). Similar results were observed in the study by de Weerd (2009), where IgG1 and IgA binding to LTA were found to be positive related to the SCC afterwards. But de Weerd also found that IgM had a negative relationship with clinical mastitis afterwards within 90 days after sampling, indicating a protective action by IgM. Unlike de Weerd's study which included details regarding mastitis incidence, the present study did not incorporate mastitis incidence and also the number of animals were more in this study.

Researches (Grabar, 1983, Tomer and Shoenfeld , 1988, Ochsenbein et. al., 1999) suggest NAb play a role in initiating an immune response. So it could be that NAbs help in reducing infections and help to maintain health. Results from the present study do not support this; cows with high NAbs do not have lower but higher SCS levels later in lactation. It maybe because the NAbs in milk may not have much role for immunity against infection, compared to the NAbs which are present in the plasma. Also, the present study used samples from cows where the health state of the animal was not known, in the sense that whether the animal had any pre-existing illness or not. All the animals present in the dataset were first parity animals, so the incidence of previous exposure to mastitis could be ruled out. But, if the animal had any other existing disease previously present, then the natural antibody levels will already be higher before the sampling was done. Studies have shown the presence of the expression of the FcRn receptor expression which is a neonatal IgG transporter, present in the small intestine of adult cows (Kacskovics, 2000).This neonatal transporter helps in carrying the antibodies to the young calves. Hence, future research can be also compared with similar analysis using plasma NAb titres of animals.

Days in lactation and calving season

Analyses showed significant effects of the days in lactation for both the mean SCS at and after and also for the standard deviation for the before and after SCS. The data used for this study did not have details regarding the infection status of the animal. Busato et.al. (2000) and Dohoo and Morris (1993) had observed for cases in subclinical mastitis that the effect of age and the days in milk of cows were important factors for subclinical mastitis. It was observed by Honkanen-Buzalski et al, (1981) that the somatic cell count increased immediately after parturition and then lowered during the first two months of lactation before increasing again towards the end of lactation. Erdem et al (2010) observed that the somatic cell counts were higher during the later stages in lactation. These changes maybe due to the effect of dilution of the somatic cells by the milk thereby leading to changes in the cell counts (Raubertas and Shook, 1982). It is also observed that there is a significant relation of the effect of season, which is the season in which the animal calved, on the standard deviation after the sampling moment. Dohoo et al, (1982) and Vliegher et al, (2004) observed higher somatic cell counts in summer compared to the other seasons, which also was related to the increased incidence of clinical mastitis during the summer period.

SCS at the sampling moment and SCS after

The analysis of the SCS at the sampling moment against the mean and standard deviations before and after SCS (table 2) also showed significant relationships which demonstrate the potential of SCS at a moment to predict the SCS later in the lactation. This shows that the SCS at the sampling moment can also be used to predict the SCS later in the lactation.

Heritability

The heritability for the NAbs and the SCS at the sampling moment which is not corrected for the NAb titre is similar as observed in a study by Wijga (2008) vusing the same data. Small differences in the estimated values maybe because of the difference in the number of animals. In a study by Buitenhuis et al (2004) in chicken a heritability for the antibody response for KLH of 0.15 was observed. A study by Siwek et al (2004) estimated heritabilies for NAb titres to LTA, LPS and KLH antigens and observed as 0.17 for LPS, 0.07 for KLH and 0.03 for LTA at 5 weeks and 0.23 for LPS, 0.42 for LTA and 0.11 for KLH at 38 weeks of age.

From the heritability estimates it was observed for the SCS after the sampling moment the average heritability was 0.30 and for the SCS at the sampling moment the average heritability was 0.08. Koivula et al (2004) in the study in Ayrshire cattle that the heritability of the log SCC changed from 0.086 in days 5-20 to 0.072 in days 301 -330; while in Holsein-Fresians the log SCC changed from 0.087 to 0.09 during the same time period. The increase of the heritability estimate for the corrected SCS after compared to the SCS at the sampling moment can be explained as a result of repeated measurements. The heritability for the SCS before the sampling moment was 0.08. It was observed that the SCS before the sampling moment had a reduced additive genetic variance compared to the SCS after the sampling moment which may have lead to a reduced heritability of the SCS before than the SCS after the sampling moment. The same pattern is observed for the standard deviation of the after SCS where the average heritability is 0.16 and the standard deviation of the before SCS is 0.01.

Conclusion

Mastitis in the dairy industry is of great economic constraint both in the large scale dairy farming as well as the small scale household systems. Present methods of mastitis detection relies on the observation of physiological or bacteriological changes evident from the milk samples which most often is known only after the presence of infection. Research shows that the use of natural antibodies for selection for disease resistance in poultry shows a positive correlation with innate immune response (Wijga, 2009)**.** The present study analyzed the relationship between NAb titres recorded at a single sampling moment with the somatic cell score measured later in the lactation. The results indicated a significant effect of the NAb titres on the SCS after the sampling moment. The trend for the SCS after was positive for increase in the Nab titre, which would not be useful for utilizing NAb titre as a protective measure against clinical mastitis infections. It was also observed that the days in lactation showed significant relations with the mean somatic cell score after the sampling moment and the standard deviation of the somatic cell score before the sampling moment. The calving season was also significant for the standard deviation of the somatic cell score after. The results, in future studies, can be compared with a similar approach with samples collected from plasma Natural antibody titres which may offer more conclusive results.

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Appendices

1.Estimates from SAS analysis the **afscs** with the LTAG1 binding NAb titre

Dependent Variable: afscs afscs

Weight: afCOUNT afCOUNT

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		1.144861767 B	2.69636097	0.42	0.6712
LTAG1		-0.066741285	0.05408558	-1.23	0.2174
sqLTAG1		0.025789903	0.00722536	3.57	0.0004
aftlacst		0.001908299	0.00050772	3.76	0.0002
ca		0.005213171	0.00600780	0.87	0.3857
ca2		-0.000003069	0.00000359	-0.85	0.3929
season	1	0.167863383 B	0.12479530	1.35	0.1788
season	2	0.062222137 B	0.11489535	0.54	0.5882
season	3	0.000000000 B	.	.	.
sire	17939	0.361853250 B	0.96510933	0.37	0.7078
sire	18099	0.265226552 B	0.98310408	0.27	0.7874
sire	18932	0.560046016 B	0.97477479	0.57	0.5657

Estimates from SAS analysis the **afscs** with the LTAG2 binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		1.970578441 B	2.72721767	0.72	0.4701
LTAG2		0.036906382	0.01123570	3.28	0.0010
sqLTAG2		0.012196616	0.00375606	3.25	0.0012
aftlacst		0.001842396	0.00051318	3.59	0.0003
ca		0.003491054	0.00608251	0.57	0.5661
ca2		-0.000002056	0.00000363	-0.57	0.5717
season	1	0.154124021 B	0.12637476	1.22	0.2228
season	2	0.052257017 B	0.11642063	0.45	0.6536
season	3	0.000000000 B	.	.	.
sire	17939	0.265170143 B	0.97623741	0.27	0.7860
sire	18099	0.182389489 B	0.99467540	0.18	0.8545

Estimates from SAS analysis the **afscs** with the LTAA binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		0.365739290 B	2.64755678	0.14	0.8901
LTAA		0.103847669	0.06390513	1.63	0.1044
sqLTAA		0.019501770	0.01041687	1.87	0.0614
aftlacst		0.001851900	0.00049923	3.71	0.0002
ca		0.005875983	0.00590364	1.00	0.3198
ca2		-0.000003395	0.00000353	-0.96	0.3360
season	1	0.052338548 B	0.12348686	0.42	0.6718
season	2	-0.003990945 B	0.11357126	-0.04	0.9720
season	3	0.000000000 B	.	.	.
sire	17939	0.658010534 B	0.94726004	0.69	0.4874
sire	18099	0.581946923 B	0.96500708	0.60	0.5466

Estimates from SAS analysis the **afscs** with the LTAM binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		0.769230461 B	2.73074453	0.28	0.7782
LTAM		-0.146507326	0.15287783	-0.96	0.3381
sqLTAM		0.038297637	0.01799405	2.13	0.0335
aftlacst		0.001873167	0.00050964	3.68	0.0002
ca		0.005529433	0.00602814	0.92	0.3592
ca2		-0.000003181	0.00000360	-0.88	0.3774
season	1	0.062908740 B	0.12528724	0.50	0.6157
season	2	-0.005730151 B	0.11524206	-0.05	0.9604
season	3	0.000000000 B	.	.	.
sire	17939	0.647552199 B	0.96980330	0.67	0.5044
sire	18099	0.601726609 B	0.98822278	0.61	0.5427

Estimates from SAS analysis the **afscs** with the LTA binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		0.281955940 B	2.68464406	0.11	0.9164
LTA		-0.019304818	0.12469545	-0.15	0.8770
sqLTA		0.034036306	0.01740607	1.96	0.0507
aftlacst		0.001924034	0.00050307	3.82	0.0001
ca		0.006610222	0.00595390	1.11	0.2671
ca2		-0.000003915	0.00000356	-1.10	0.2715
season	1	0.112368304 B	0.12352555	0.91	0.3632
season	2	0.038924652 B	0.11386706	0.34	0.7325
season	3	0.000000000 B	.	.	.
sire	17939	0.401943413 B	0.95709765	0.42	0.6746
sire	18099	0.315616883 B	0.97510087	0.32	0.7462

Estimates from SAS analysis the **afscs** with the KLH binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		1.293649144 B	2.75123776	0.47	0.6383
KLH		-0.002663247	0.18792685	-0.01	0.9887
sqKLH		0.020409023	0.02336263	0.87	0.3825
aftlacst		0.001930197	0.00051297	3.76	0.0002
ca		0.003478617	0.00606341	0.57	0.5663
ca2		-0.000001903	0.00000362	-0.53	0.5996
season	1	0.093814590 B	0.12653476	0.74	0.4586
season	2	0.019227000 B	0.11635531	0.17	0.8688
season	3	0.000000000 B	.	.	.
sire	17939	0.536660536 B	0.97735266	0.55	0.5830
sire	18099	0.482825186 B	0.99589993	0.48	0.6279

Estimates from SAS analysis the **afscs** with the LPS binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		1.475124503 B	2.74120120	0.54	0.5906
LPS		0.097294083	0.09961945	0.98	0.3289
sqLPS		-0.003789549	0.01561689	-0.24	0.8083
aftlacst		0.001859438	0.00051596	3.60	0.0003
ca		0.003470055	0.00610324	0.57	0.5698
ca2		-0.000001950	0.00000365	-0.53	0.5930
season	1	0.090183316 B	0.12676979	0.71	0.4770
season	2	-0.000469065 B	0.11671820	-0.00	0.9968
season	3	0.000000000 B	.	.	.
sire	17939	0.489013227 B	0.98226400	0.50	0.6187
sire	18099	0.446264962 B	1.00083243	0.45	0.6557

Estimates from SAS analysis the **afscs** with the PGN binding NAb titre

Parameter		Estimate	Standard Error	t Value	Pr > |t|
Intercept		2.408432375 B	2.65080742	0.91	0.3637
PGN		0.033981524	0.15255249	0.22	0.8238
sqPGN		0.020656209	0.01261635	1.64	0.1018
aftlacst		0.001699910	0.00048936	3.47	0.0005
ca		0.000858606	0.00578415	0.15	0.8820
ca2		-0.000000642	0.00000346	-0.19	0.8528
season	1	0.147769074 B	0.11985829	1.23	0.2178
season	2	0.101011476 B	0.11058711	0.91	0.3612
season	3	0.000000000 B	.	.	.
sire	17939	0.229609169 B	0.92827578	0.25	0.8047
sire	18099	0.202645903 B	0.94569702	0.21	0.8304

Estimates from SAS analysis the **afscs** with the SCS at the sampling moment (SCSsample)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SCSsample	1	139.541857	139.541857	94.98	<.0001
sqSCSsample	1	8.306682	8.306682	5.65	0.0176
aftlacst	1	8.295259	8.295259	5.65	0.0176
ca	1	0.948619	0.948619	0.65	0.4218
ca2	1	0.760921	0.760921	0.52	0.4718
season	2	4.065285	2.032643	1.38	0.2510
sire	98	183.175344	1.869136	1.27	0.0421
herd	389	1024.894242	2.634690	1.79	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.194680003 B	1.91510803	-0.10	0.9190
SCSsample	0.728704572	0.07477023	9.75	<.0001
sqSCSsample	-0.020512970	0.00862669	-2.38	0.0176
aftlacst	0.000859417	0.00036167	2.38	0.0176
ca	0.003422605	0.00425932	0.80	0.4218

2. Phenotypic correlations

Correlation between **afscs** (mean of nat. log. of the scc after the sampling moment) and NAb titres

Correlation Matrix

	afscs	LTAG1	LTAG2	LTAM	LTAA	KLH	LPS	LTA	PGN
afscs	1.0000	0.1836	0.1125	0.1839	0.2212	0.1393	0.1055	0.2209	0.2847
LTAG1	0.1836	1.0000	0.5110	0.3321	0.3002	0.2025	0.1836	0.5153	0.3589
LTAG2	0.1125	0.5110	1.0000	0.1654	0.2019	0.0786	0.0742	0.2830	0.3234
LTAM	0.1839	0.3321	0.1654	1.0000	0.6748	0.6402	0.4567	0.5886	0.2732
LTAA	0.2212	0.3002	0.2019	0.6748	1.0000	0.5145	0.3609	0.5508	0.3122
KLH	0.1393	0.2025	0.0786	0.6402	0.5145	1.0000	0.4916	0.4944	0.2448
LPS	0.1055	0.1836	0.0742	0.4567	0.3609	0.4916	1.0000	0.4019	0.2010
LTA	0.2209	0.5153	0.2830	0.5886	0.5508	0.4944	0.4019	1.0000	0.4099
PGN	0.2847	0.3589	0.3234	0.2732	0.3122	0.2448	0.2010	0.4099	1.0000

Correlations with the nat. log of mean scc at the sampling moment (**SCSsample**) and the NAb titres

Correlation Matrix

	SCSsample	LTAG1	LTAG2	LTAM	LTAA	KLH	LPS	LTA	PGN
SCSsample	1.0000	0.2192	0.1249	0.2542	0.3159	0.2248	0.1788	0.3182	0.3544
LTAG1	0.2192	1.0000	0.5126	0.3329	0.2993	0.2046	0.1887	0.5130	0.3551
LTAG2	0.1249	0.5126	1.0000	0.1733	0.2057	0.0831	0.0773	0.2849	0.3197
LTAM	0.2542	0.3329	0.1733	1.0000	0.6742	0.6456	0.4601	0.5900	0.2745
LTAA	0.3159	0.2993	0.2057	0.6742	1.0000	0.5159	0.3670	0.5524	0.3162
KLH	0.2248	0.2046	0.0831	0.6456	0.5159	1.0000	0.4962	0.4992	0.2512
LPS	0.1788	0.1887	0.0773	0.4601	0.3670	0.4962	1.0000	0.4103	0.2134
LTA	0.3182	0.5130	0.2849	0.5900	0.5524	0.4992	0.4103	1.0000	0.4182
PGN	0.3544	0.3551	0.3197	0.2745	0.3162	0.2512	0.2134	0.4182	1.0000

Correlation between **b4scs** (mean of nat. log. of the scc before the sampling moment) and NAb titres

Correlation Matrix

	b4scs	LTAG1	LTAG2	LTAM	LTAA	KLH	LPS	LTA	PGN
b4scs	1.0000	0.1874	0.0795	0.2103	0.2568	0.1644	0.1397	0.2381	0.2905
LTAG1	0.1874	1.0000	0.5122	0.3366	0.3010	0.2040	0.1879	0.5171	0.3605
LTAG2	0.0795	0.5122	1.0000	0.1698	0.2075	0.0795	0.0783	0.2858	0.3241
LTAM	0.2103	0.3366	0.1698	1.0000	0.6742	0.6424	0.4608	0.5891	0.2697
LTAA	0.2568	0.3010	0.2075	0.6742	1.0000	0.5138	0.3660	0.5511	0.3127
KLH	0.1644	0.2040	0.0795	0.6424	0.5138	1.0000	0.4945	0.4974	0.2430
LPS	0.1397	0.1879	0.0783	0.4608	0.3660	0.4945	1.0000	0.4087	0.2080
LTA	0.2381	0.5171	0.2858	0.5891	0.5511	0.4974	0.4087	1.0000	0.4142
PGN	0.2905	0.3605	0.3241	0.2697	0.3127	0.2430	0.2080	0.4142	1.0000

Correlations with the standard deviation of scs before sampling moment (**b4stdscs**) and the NAb titres.

Correlation Matrix

	b4stdscs	LTAG1	LTAG2	LTAM	LTAA	KLH	LPS	LTA	PGN
b4stdscs	1.0000	0.0668	0.0342	0.0799	0.0761	0.0378	0.0596	0.0761	0.1196
LTAG1	0.0668	1.0000	0.5132	0.3339	0.3009	0.2022	0.1876	0.5174	0.3600
LTAG2	0.0342	0.5132	1.0000	0.1706	0.2074	0.0809	0.0776	0.2863	0.3239
LTAM	0.0799	0.3339	0.1706	1.0000	0.6748	0.6413	0.4618	0.5899	0.2679
LTAA	0.0761	0.3009	0.2074	0.6748	1.0000	0.5144	0.3668	0.5511	0.3112
KLH	0.0378	0.2022	0.0809	0.6413	0.5144	1.0000	0.4960	0.4980	0.2425
LPS	0.0596	0.1876	0.0776	0.4618	0.3668	0.4960	1.0000	0.4093	0.2082
LTA	0.0761	0.5174	0.2863	0.5899	0.5511	0.4980	0.4093	1.0000	0.4143
PGN	0.1196	0.3600	0.3239	0.2679	0.3112	0.2425	0.2082	0.4143	1.0000

Correlations with standard deviation of scs after sampling moment (**afstdscs**) and the NAb titres.

Correlation Matrix

	afstdscs	LTAG1	LTAG2	LTAM	LTAA	KLH	LPS	LTA	PGN
afstdscs	1.0000	0.0370	0.0329	-0.0358	-0.0291	-0.0286	0.0017	0.0314	0.0596
LTAG1	0.0370	1.0000	0.5180	0.3256	0.2931	0.1915	0.1794	0.5120	0.3545
LTAG2	0.0329	0.5180	1.0000	0.1672	0.2014	0.0775	0.0789	0.2833	0.3179
LTAM	-0.0358	0.3256	0.1672	1.0000	0.6706	0.6329	0.4508	0.5803	0.2709
LTAA	-0.0291	0.2931	0.2014	0.6706	1.0000	0.5101	0.3528	0.5442	0.3121
KLH	-0.0286	0.1915	0.0775	0.6329	0.5101	1.0000	0.4877	0.4859	0.2391
LPS	0.0017	0.1794	0.0789	0.4508	0.3528	0.4877	1.0000	0.4002	0.2054
LTA	0.0314	0.5120	0.2833	0.5803	0.5442	0.4859	0.4002	1.0000	0.4076
PGN	0.0596	0.3545	0.3179	0.2709	0.3121	0.2391	0.2054	0.4076	1.0000

3. Table 2. Relationship between NAb binding antigens which showed significant relation and SCS at the sampling moment