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The Relationship Between Milk Somatic Cell Count and Blood Concentrations of Haptoglobin and Serum Amyloid A In Dairy Cows.

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Abstract: In this study the relationship between somatic cell count (SCC) and blood concentrations of haptoglobin (Hp) and serum amyloid A (SAA) were investigated to determine the ability of these proteins as a reliable indicators for monitoring udder health and subclinical mastitis with SCC in dairy cows. The results showed a positive significant correlation ($P<0.01$) between SAA and SCC but this correlation for Hp and SCC were not significant. There were also a statistically significant difference between two defined group (SCC< 4000000 and SCC≥ 4000000) for SAA. So, detectable level of SAA were more common at high SCC.

Key words: Serum Amyloid A, Haptoglobin, Somatic Cell Count, Dairy Cows

INTRODUCTION

Mastitis is one of the most causes of economic losses in dairy herds (Radostits *et al*., 2007). This economic losses is due to reduction in milk production, discarded milk and treatment costs (Seegers *et al*., 2003). Bovine mastitis results from inflammation of the mammary gland. The severity of the inflammation can be classified into clinical, subclinical and chronic forms (Gronlund *et al*., 2003; Radostits *et al*., 2007). Clinical mastitis is often easy to detect, while subclinical is difficult to detect due to absence of visible changes in the appearance of the milk or udder. It is said that one third of one half of cows have intra mammary infections, with the vast majority of infection being subclinical. This form of mastitis is accompanied by an increase in somatic cell count (SCC) in milk and reduction in milk yield, the extent and duration of which depend on the causative pathogen and effectiveness of host defence mechanisms. Total milk losses from quarter affected with subclinical mastitis have been estimated to range from 10-26% (Radostits *et al*., 2007).

SCC is the standard procedure for detecting of subclinical mastitis in cattle and it has been widely accepted and used to indicate the health status of bovine mammary gland, the quality of milk for human consumption and the hygiene conditions of dairy farms (Gruys *et al*., 2005). Several researchers have focused on the relation of acute phase proteins (APPs) with mastitis as a rapid test for detection of subclinical mastitis (Eckersall *et al*., 2001; Mcdonald *et al*., 2001; Gronlund *et al*., 2005; Nazifi *et al*., 2008a). The APPs are reactant synthesized during an acute phase response (APR) against infection, inflammation, trauma, tissue injury (Peterssen *et al*., 2004; Gruys *et al*., 2005). Haptoglobin (HP) and Serum Amyloid A (SAA) are the major acute phase proteins and they have been shown to be good indicators to discriminates between acute and chronic inflammatory conditions in cattle (Horadagoda *et al*., 1999).

To our knowledge the correlation between SAA and HP and SCC has not yet been fully considered.. The aim of this study was to determine any relations between these acute phase proteins (Serum Amyloid A, Haptoglobin) and SCC in dairy cows in order to asses the ability of this parameters as a part of monitoring of udder health in dairy herds.

MATERIAL AND METHODS

The study was done in 10 commercial dairy herds, located in Tehran province of Iran. A total of 110 cows without any history of recent illness or off-feed episodes and clinical mastitis between 50-150 days in milk were selected. Samples of milk were collected of each cows (composite milk) after teat cleaning with ethanol 70%. First streams of milk were discarded and then 30-40 mililiter (mL) of milk was collected aseptically from each cow into sterile tubes. Milk samples were stored at 4°C until analysis (about 2 hours). Blood samples were collected by tail venipuncture from each cow. Samples were collected in blank and heparinized 10-mL evacuated tubes for serum and plasma collection respectively. The serum in blank evacuated tubes were allowed to clot at room temperature (19°C) for 45 minutes (min) and then centrifuged at 1900× *g* for 15 min and then serums was aspirated and stored at -20°C until it was used for Hp analysis. Plasma was harvested by

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centrifuging heparinized evacuated tubes at 1900×*g* for 15 min and was stored at -20°C until it was analysed for serum amyloid A. The direct microscopic somatic cell count was used for determination of SCC. Serum concentrations of Hp and plasma concentrations of SAA were determined using ELISA kit (BIO-X and Tri-Delta Diagnostics Inc., respectively) according to the manufacturer´s instructions.

Statistical Analysis:

Data were analysed by Pearson Correlation Test for SAA, HP and SCC using SPSS19. For analysis of the effects of SCC on blood SAA and HP concentrations, samples also were analysed by Independent Smples T Test on the basis of defined grouping by low (SCC< 200000 cells/ml) or high (SCC≥ 200000 cells/ml) For Hp and SAA.

RESULT AND DISCUSSION

A total of 110 cows were included in this study. The mean values of HP, SAA and SCC are recapitulated in table 1.

Table 1: The mean values of Lac, DIM, HP, SAA and SCC in the herds.

Item	Mean ± SD
Lac	3.48 ± 1.74
DIM	99.31 ± 27.81
Haptoglobin (mg/ml)	0.11 ± 0.40
Serum Amyloid A (ng/ml)	108.73± 72
Somatic cell Count (1000cells/ml)	477.37 ± 1228

SD= standard deviation
Lac= lactation
DIM= days in milk

The results showed a positive significant correlation (*P*< 0.01) between SAA and SCC (r= 0.305, *P*= 0.002) but the correlation between HP and SCC were not significant (r= 0.07, *P*= 0.41). A significant correlation (*P*<0.01)

also were observed between SAA and HP. There was a significant difference (*P*< 0.001) in SCC between two groups (SCC< 200000 and SCC≥ 200000). There were no significant difference between two defined group (SCC< 200000 and SCC≥ 200000) for Hp and SAA but when the mean values of SCC approached to 4000000, the difference for SAA in defined group (SCC< 4000000 and SCC≥ 4000000) became significant (Fig.1)

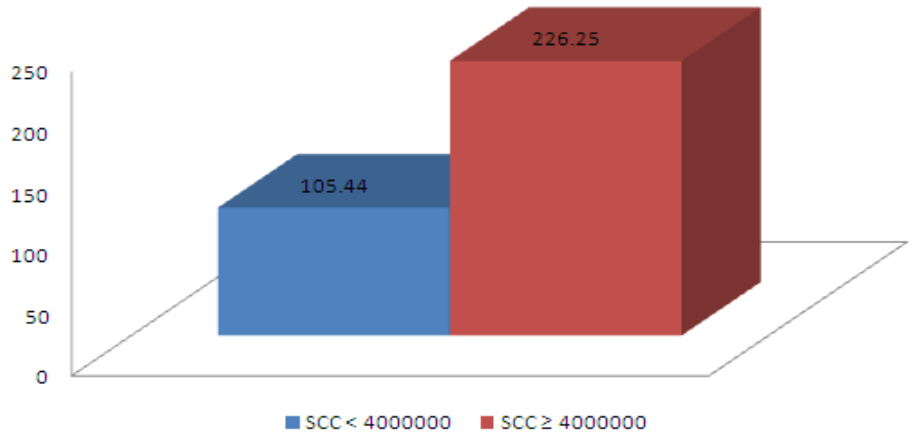

Fig. 1: The mean values of SAA (ng/ml) in cows with SCC< 4000000 and SCC≥ 4000000.

Discussion:

Some researchs have suggested that acute phase protein may have diagnostic value in identifying cows with subclinical and clinical mastitis (Eckersall *et al*., 2001; Gronlund *et al*., 2005). It has been shown that concentrations of SAA and HP in blood and milk increases in cows with mastitis (Akerstedt *et al*., 2007; Eckersall *et al*., 2007; Nielsen *et al*., 2004). Colla *et al*., (2011) have stated that plasma concentration of Hp was higher (*P* <0.05) in the group of mastitic cows comparing the other two groups (healthy and subclinical mastitis group) but no difference was found between healthy cows and cows with subclinical mastitis. They also found a

significant correlation between SCC and Hp (r= 0.357) showing that SCC may be determinant for the increment of blood Hp concentration, especially in cows with high values of SCC. In contrast of that, In the present study this correlation for Hp and SCC was not statistically significant but there was a statistically significant correlation (P< 0.01) between SAA and high values of SCC (r=0.305, *P*=0.002). Horadagoda *et al*., (1999) have reported that serum values of SAA seem to be more sensitive marker for acute inflammation than Hp values. Some researchs have indicated that the concentrations of major acute phase proteins (APPs) like Hp and SAA in the milk (M-SAA) in cases of mastitis is much higher than in blood concentrations (Tabrizi *et al*., 2008; Safi *et al*., 2009). Akerstedt *et al*., (2007) and Kovac *et al*., (2011) have reported significant relationships between Hp, M-SAA and SCC at quarter and cow composite milk level that are in agreement with the results for SAA in blood but not for Hp in our study. Although some authors have stated that high M-SAA in relation to low SCC may represent false negative result for SCC (O' Mahony *et al*., 2006). This result that there were no significant correlation between Hp and SCC in the present study may be due to absence of clinical mastitis in the cows. In the study of Zeng *et al*., (2009) there were a greater of blood concentrations of haptoglobin in cows with clinical mastitis than in cows with either subclinical intra mammary infection or all healthy quarters. Additionally,the differences in response between SAA and Hp may be due to high sensitivity of SAA to inflammatory process than Hp (Humblet *et al*., 2006). Because the cytokines that are involved in initiating the synthesis of these acute phase proteins are different. Either interlukin-6 or tumor necrosis factor-α is required for the synthesis of SAA, but both of them are required for Hp synthesis and there are a different in acute phase response (Alsemgeest *et al*., 1996; Plaizier *et al*., 2009). It seems that Hp may be the sensitive indicator in the chronic inflammatory process.

In conclusion, detectable level of APPs are more common at high SCC. It can be possible to differentiate between acute and chronic disease like mastitis or others by using these proteins. However, further investigations are needed on a larger sample size of cows for these proteins and other biomarkers in blood and milk in relation to udder health and monitoring the status of the cows and herds for being in the good condition.

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