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Original article

Evaluation of acute phase proteins in clinically healthy dairy cows in perinatal period and during lactation

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Abstract

The estimation of acute phase proteins (APP), which are recognized as inflammation markers is a good method for animal health monitoring. Several factors such as obesity, age and sex are also known to modulate APP status. We evaluated the influence of pregnancy and lactation in 65 clinically healthy dairy Holstein-Friesian dairy cows, 2nd÷4th lactation, chosen from 3 different dairy farms located in South West part of Poland. Bovine C-reactive protein (CRP), haptoglobin and fibrinogen were assayed using commercial ELISA kits. The highest values of CRP and haptoglobin were observed in cows during the first month after calving. The highest concentrations of fibrinogen was found in a group of cows prior to expected date of parturition and the level of this protein in blood plasma was decreasing during lactation. The significant differences of analyzed APPs among cows before delivery, during first month after calving and in lactation (1-3 months after delivery) suggested that factors like pregnancy and stage of lactation would have an influence on their concentration.

Key words: cows, acute phase proteins, perinatal period, lactation

Introduction

Through generations cattle were selected focused on increasing productivity (high volumes of milk), but now health and animal welfare also became very important. The maintenance of a good health is the most essential requirement having strong impact on cattle welfare. A key role of inflammation in several infectious and also metabolic diseases has been documented. Cytokines produced during inflammation such as necrosis factor alpha (TNFα), interleukin 1β and interleukin 6 act through many signaling cascades producing often similar responses of organism. Cytokines are acting as messengers between the local site of inflammation (injury) and liver in which the synthesis of the acute phase proteins take place. Most of these proteins have multiple functions and targets. They are non-specific inflammation markers and can

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be used in the evaluation of an individual cow, as well as a heard health status, and also for early disease surveillance. However, such factors like obesity, age and sex of animals were recognized to influence APP production and concentration in the blood (Maffei et al. 2009, Christoffersen et al. 2015).

Acute phase response is considered to be a dynamic process providing an early nonspecific defense mechanism against injury, infection or inflammation before response of specific immunity. Very important positive APPs are haptogobin, C-reactive proteins, and serum amyloid A that possess scavenging activities to bind metabolites released during cellular degradation. Another positive APP is fibrinogen which increased concentration in blood serum is a result of cytokine stimulation of blood coagulation system. The serum level of these proteins increases within few hours after pathological stimulus and this higher concentration is detectable for several days. Systemic inflammatory reactions also include decreased serum concentration of some others plasma proteins (negative APP) like lipoproteins, retinol-binding protein, transferrin and blood albumin (Gruys et al. 2005). Estimation of positive APP's serum concentration may provide an alternative means for animal health status monitoring. They are not suitable for specific diagnosis, similarly to fiver, but might be useful for determination when spreading of a disease is ongoing. There is notable variation in the response of APP among different species (Khan et al. 1994, Eckersall 1995). Review of the role and APP application in different animals (cattle, horses, pigs) was given by Peterson et al. (2004) and in ruminants by Tothova et al. (2014). Lee et al. (2003), who have shown that serum C-reactive protein (CRP) is a good marker for heard health status and may be useful in early surveillance of diseases in dairy cows. They observed that diseases and adverse environmental conditions induce much higher increase of CRP than stress or lactation. APP might be also used in cattle for discrimination between acute and chronic inflammation (Horadagoda et al. 1999, Burfeind et al. 2014). Stepwise decrease of APP level was observed in dairy cows during treatment of limb diseases (Jawor et al. 2008). Another APP is haptoglobin which was recognized as a marker of inflammation in dairy cows, however increase of its concentration was noticed also in a week after calving (Humblet et al. 2006). These authors also observed increased haptoglobin level in ca. 10% of healthy cows. In case of subclinical endometritis affecting dairy cows in early postpartum period the increased concentration of proinflamatory cytokines and haptoglobin were found not only in serum, but also in the uterine washings (Brodzki et al. 2015). It was shown by Schroedl et al. (2014), that gut microflora may also modulate the synthesis of APPs such as haptoglobin and C-reactive protein.

Fibrinogen concentration is also used to study human and animal health status. The increase of fibrinogen in cows plasma may accompany different sort of tissue inflammation and/or destruction (McSherry et al. 1970). APPs are alternative biomarkers of mastitis and may increase before macroscopic changes in the milk. However, in subclinical mastitis milk is much better than blood plasma for APP estimation (Safi et al. 2009). It is also known that changes in the oxidation state of organism, cell metabolism and stress may have strong influence on blood APPs value. That's why pregnancy, delivery and lactation may have strong influence on APPs production, and variation among reactions of particular proteins may occur. There is very limited information in the available literature about changes of APPs status in healthy cows in perinatal period and lactation.

The aim of this study was to estimate different APP in the blood of clinically healthy dry cows, in cows before parturition and during lactation as well as analysis of observed changes of APP concentrations.

Materials and Methods

The study was performed on 65 clinically healthy dairy Holstein-Friesian cows chosen from 3 dairy cows farm. The farms were located in South West Poland with 200÷300 cows fed a TMR diet and housed in an open, free-stall barns with concrete floor covered with straw and rubber mats. Lactating cows were milked twice a day in parallel parlor system with 8 milking units. Duration of lactation period was on average 300±15d and the milk yield calculated per lactating cow was 8000÷9000 l. Blood samples were taken from jugular vein to EDTA containing tubes, before parturition, in early lactation (0-1 month after calving) and 1-3 months after parturition. Blood collection took place in the morning within 1h. Samples were placed on ice and centrifuged for 10 min at 3000g. Obtained plasma was stored at -80ºC until analysis.

APP levels in blood plasma were evaluated using ELISA commercial kits: bovine C-reactive protein, bovine Fibrinogen and bovine Haptoglobin kits were produced by GenxBio Health Sciences Pvt. Ltd. (Delhi, India).

Statistical analysis was performed using software that used ANOVA method. We checked whether certain independent variables (factors) affect the value of the dependent variable (the variable being tested and measured). In our case we examined the statistical significance of the experiment, in which we measured the value of acute phase proteins in a herd of cows.

Table 1. Average means of acute phase proteins concentration in cows plasma during perinatal period.

Acute Phase Proteins	Before delivery n=25	0-1 month after delivery n=15	1-3 months after delivery n=25
C-Reactive Pr. mg/l	105.0 ± 9.1[a]	126.8 ± 15.3[b]	104.3 ± 15.1[a]
Haptoglobin mg/l	109.4 ± 12.3[a]	139.8 ± 9.8[b]	128.9 ± 12.7[c]
Fibrynogen g/l	6.52 ± 0.99[a]	5.66 ± 1.09[b]	5.02 ± 0.87[c]

Means ± SD
Means in rows with unlike subscript letters differ at p≤0.05 level

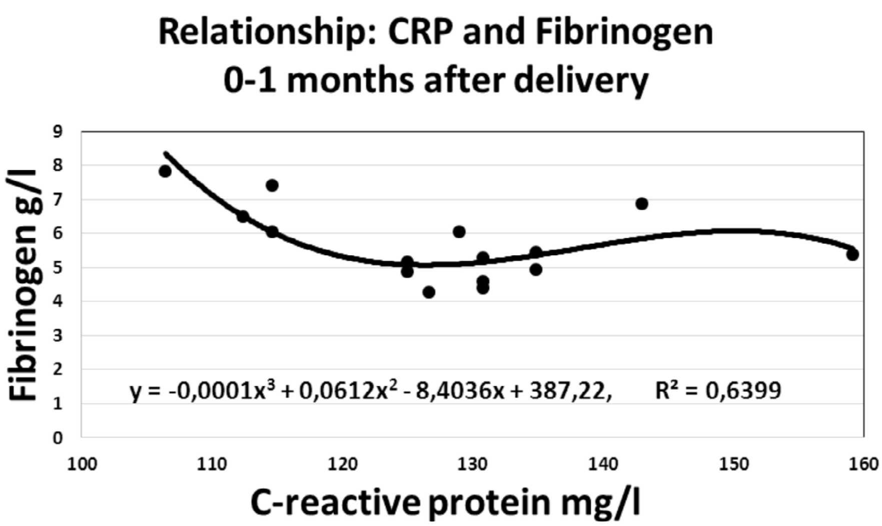

Fig. 1. The correlation of fibrinogen and CRP values in blood plasma of cows during the first month after calving.

The independent variable in statistical analysis was moment of sampling. Blood samples were collected from cows before delivery, during the first month after delivery and continued after one-three months after calving.

Results

APPs results in blood plasma of investigated cows were presented in Table 1. In the investigated cows the concentration of CRP was significantly higher in the first month after calving. Values of this APP in pregnant (last trimester) cows and lactating cows (1-3 months after delivery) was 21% lower comparing to rest of cows. The lowest concentration of haptoglobin was found in cows just before delivery. In cows during the first month after calving it was observed the increase of haptoglobin concentration (28%). In peak lactation (1-3 months after calving) slight, but still significant decrease of APP value was noted. The highest concentration of fibrinogen was found in cows before delivery whereas after calving significant decrease was observed.

A strong correlation between fibrinogen and CRP values was noticed in cows after delivery (0-1 month) (Fig. 1). R^2 coefficient value was calculated (R^2=0.64) as a measure for the goodness of fit in nonlinear model of these relations. Weak correlations (R^2=0.1÷0.2) were also found between fibrinogen and CRP in cows before delivery and in lactating cows 1-3 months after calving. Correlation between haptoglobin and CRP values was observed in cows during first month after calving (R^2=0.32).

Discussion

The estimations of metabolites, hormones and different blood proteins for assessment of single animal and/or herd health during transition period has been used for many years. Several studies are showing that during the period before calving and few weeks after parturition cows suffer from stress-related problems and often have an inflammation associated with injury or infection. Many cows experience a period of negative energy balance after calving, which is caused by limited feed intake and higher energy requirements due to increased milk production (Lu et al. 2013). This period is also connected with environmental and management changes (e.g. milk parlor, social regrouping). All these factors lead to the increase of non-specific inflammation markers in the blood depending on the degree of health complication and environmental stressors. The evaluation of APPs as markers of stress and inflammation could be used for identification of cows at an increased risk of diseases or performance problems. The most widely studied APP in cattle is haptoglobin which is considered to be a very useful marker for the early identification of not only common transition cows' disorders e.g. mastitis or metritis, but also e.g. respiratory infections (Burfeind et al. 2014, Prohl et al. 2015). Haptoglobin is binding free haemoglobin in the blood plasma released from erythrocytes. The synthesis of this APP is stimulated by stress conditions, what is in agreement with study of Argente et al. (2014) who has shown a positive correlation between cortisol level and haptoglobin concentration in female rabbits, however statistical significance of this correlation was relatively low (p=0.09). Eckesall et al. (2001) found that the diagnostic value in differentiation between healthy cow and animals suffering clinical mastitis gave sensitiveness and specificities on the level of 82 and 94%, respectively. The ability of haptoglobin to differentiate between acute and chronic inflammation was observed in cattle by Horadagoda et al. (2013) with efficiency of 73%. We found 28% higher levels of haptoglobin in cows during the first month after delivery comparing with values before calving. This increase was also noticed in a study performed by Humblet et al. (2006) in healthy cows during the first week after calving. Observed long lasting increase in haptoglobin level might be explained by approximately longer half-life of this APP (Jahoor et al. 1996), and by the physiological increase of APPs after parturition. During this period mild inflammations may take place without a clinical manifestation. The higher value of haptoglobin concentration observed in blood plasma was related to the similar increase of C-reactive protein. Relationship of these APPs after calving was supported by the observed significant correlation (R^2=0.32). Examined cows during one month period following calving had 21% higher concentration of C-reactive protein comparing to cows before parturition. It is in agreement with results presented by Lee al. (2003) showing that plasma C-reactive protein concentration is related to different kind of stress (poor health, high lactation, blood collection).

Third analyzed APP in our experiment was fibrinogen which is a fibrin precursor and take also part in localization of tissues in which inflammatory process take place. Plasma fibrinogen is a good indicator of an inflammatory reactions, but data regarding this protein in clinically healthy cattle is very limited. However, Laven et al. (2004) in a study concerning hoof haemorrhages in primiparous cows did not observe relation between these haemorrhages and fibrinogen concentration. This observation suggests that hoof horn haemorrhages may not be caused by endotoxicosis. The changes in fibrinogen concentration may be related to pregnancy and delivery. It is known that during pregnancy the activation of blood coagulation panel takes place, and these changes in blood coagulation system, including fibrinogen concentration, are normalized within one month after delivery (Hellgren 2003). In our study the highest concentrations of fibrinogen were observed in cows just before calving. Then, during lactation, these values declined and this decrease was statistically significant. Strong correlation was observed in cows after delivery (0-1 month) between fibrinogen and CRP values.

Obtained results suggest that not only inflammations but also physiological factors such as pregnancy, delivery and/or state of lactation may have a significant impact on APPs values in the blood plasma of dairy cows. It would be worth in the future to check whether there is a relationship assessing the animal health status obtained using acute phase proteins method relatively to other indicators, such as milk yield, length of lactation or others.

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