The association between indicators of inflammation and liver variables during the transition period in high-yielding dairy cows: An observational study

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ABSTRACT

During the transition period, cows are confronted with infectious and inflammatory challenges leading to an acute phase response (APR) marked by increased hepatic synthesis of positive acute phase reactants (+AP) and a decrease in negative acute phase reactants (−AP). The aim of this study was to quantify the APR in 21 high-yielding dairy cows studied from 9 days before until 42 days after calving, and to assess the association between the APR, disease incidence and indicators of liver function. Repeated blood samples were analyzed for −AP (retinol, albumin, cholesterol), +AP (haptoglobin, caeruloplasmin), paraoxonase, and liver-associated variables (aspartate aminotransferase, γ-glutamyl transferase, bilirubin).

All cows displayed postpartum decreases in −AP and paraoxonase, and increases in +AP and liver variables. When retrospectively categorized, cows presenting a stronger −AP decline displayed higher +AP and liver variables, and a higher disease incidence compared to cows with a milder decline. Altogether, typical changes in −AP and +AP identify the transition period as a time of increased inflammatory load. Group differences in liver variables suggest that a more severe APR may be associated with altered liver function. However, no causal relationship can be proven based on this observational dataset, and results should be interpreted cautiously.

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Introduction

The transition period, roughly stretching from 3 weeks before to 3 weeks after parturition (Mulligan and Doherty, 2008), is a difficult period for high-yielding dairy cows, and is characterized by a high incidence of metabolic, infectious, and reproductive disorders (Goff and Horst, 1997). Adequate adaptation of metabolic pathways in the liver to drastically changing energy needs is particularly important for a successful transition period (Drackley et al., 2001). For instance, glucose requirements for milk production can only be met through strong up-regulation of hepatic gluconeogenic output (Greenfield et al., 2000; Drackley et al., 2001). The liver also has to process the increased flow of non-esterified fatty acids resulting from negative energy balance post partum (pp) (Goff and Horst, 1997). Remarkably, over 50% of fresh high-yielding dairy cows suffer from subclinical hepatolipidosis (Jorritsma et al., 2001), showing that liver function is seriously tested during early lactation.

Although most cows cope with these challenges, the line between ‘physiological’ and ‘pathological’ changes is thin. Any source of additional strain on liver function further increases the risk of transition period disorders (Goff and Horst, 1997; Drackley et al., 2001; Bobe et al., 2004). In this context, inflammatory conditions such as endometritis and mastitis seem particularly important (Katoh, 2002; Drackley et al., 2005). Inflammation evokes the release of tumour necrosis factor-α and interleukin-1 and -6 from leukocytes, in turn triggering the acute phase response (APR) (Petersen et al., 2004). Inflammation is characterized by the upregulation of the hepatic synthesis of positive acute phase reactants (+AP) including haptoglobin, caeruloplasmin and serum amyloid-A, while a reduction or shift takes place in the synthesis of negative acute phase reactants (−AP) such as transferrin, albumin, retinol binding protein (RBP) and apolipoproteins (Petersen et al., 2004; Gruys et al., 2005; Bionaz et al., 2007).

Many of these changes may be beneficial for the host; for instance, a decrease in transferrin synthesis reduces iron availability for bacteria (Hirvonen et al., 1999; Petersen et al., 2004; Gruys et al., 2005). However, the APR may also have undesirable consequences for the transition period (Goff and Horst, 1997; Gruys et al., 2005). For example, lowered retinol, apolipoprotein and
paraoxonase (PON) concentrations may impair immunocompetence and increase the risk of lipotoxicity (Goff and Stabel, 1990; Feingold et al., 1998; Katoh, 2002). Indeed, low –AP levels have recently been associated with decreased liver function, reproductive performance and milk yield (Bionaz et al., 2007; Bertoni et al., 2008).

Despite extensive research in this field, the complex transition biology of the dairy cow is not yet completely understood (Goff and Horst, 1997; Drackley, 1999; Drackley et al., 2001). In particular, the dynamics of +AP and –AP concentrations around parturition and their clinical relevance for transition period performance require further elucidation (Humblet et al., 2006; Bertoni et al., 2008). The aims of this study were (1) to describe in detail the pattern of –AP and +AP changes during the transition period in high-yielding dairy cows, and (2) to investigate the association of APR with disease incidence and liver variables.

**Material and methods**

For this study, a series of blood samples obtained from 21 cows in a previous experiment (Bossaert et al., 2008) were used. Blood samples were obtained on regular time intervals before and after calving. Health status and daily milk yield were recorded. A concise description of experimental procedures is provided below. More detailed information about the protocols, the animals, their housing, feeding, metabolic profiles, and milk yield is presented in Bossaert et al. (2008).

**Animals**

All experimental procedures were approved by the Ethical Committee of the Faculty of Veterinary Medicine (Ghent University, Merelbeke, Belgium). Twenty-one multiparous Holstein–Friesian cows with a high genetic merit for milk yield, housed in a cubicle yard and milked by a voluntary milking system, were studied from 14 days prior to the expected calving date until 42 days pp.

**Blood sampling**

Blood samples were obtained from the coccygeal vein 9 days prior to the expected calving date and on days 1, 8, 15, 21, 28, and 42 pp in gel-coated blood tubes and blood tubes containing sodium fluoride (Venoject, Terumo). Samples were centrifuged and the plasma or serum was stored at −80 °C.

**Analyses**

Samples were analyzed for retinol (µg/dL), albumin (g/L), total cholesterol (mmol/L), paraoxonase (PON; U/mL), caeruloplasmin (µmol/L), haptoglobin (g/L), total bilirubin (µmol/L) and aspartate aminotransferase (AST; U/L), γ-glutamyl transferase (GGT; U/L) using commercially available kits (Cobas, Roche Diagnostics; Wako Chemicals; Instrumentation Laboratory). All methods of analysis are described in more detail elsewhere (Bionaz et al., 2007; Bertoni et al., 2008; Bossaert et al., 2008).

**Data handling and groups**

For each cow, a quantitative estimation of the APR was made by computing the liver activity index (LAI) based on –AP concentrations, as described by Bertoni et al. (2008). Briefly, albumin, cholesterol, and retinol concentrations on 8, 15, and 28 days pp were converted into units of standard deviation from the group’s average. The final LAI for a cow was the arithmetical mean of the three computed indices at the three time points. The cows were retrospectively divided into two groups based on their LAI: Group 1 (LAI < 0; n = 10) and Group 2 (LAI > 0; n = 11). All variables (–AP and +AP, paraoxonase, liver variables, disease incidence) were analyzed by time (categorical: −9, 1, 8, 15, 21, 28 and 42 days pp) and LAI (categorical: Group 1 and Group 2).

**Statistical models**

Analyses were performed using SPSS 17.0 and SAS Enterprise Guide 4.1. A P-value > 0.10 was considered a tendency; a P-value < 0.05 was considered statistically significant. Normality of data was tested by the Kolmogorov–Smirnov method. In case of non-normality, exponential transformation with a Box–Cox macro was carried out. All variables were analyzed as repeated measurements in a mixed ANOVA model to investigate the main effects of time, group, and time × group interaction, with AR(1) as the covariance structure. Independence of LAI group and disease incidence was investigated by computing Pearson’s Chi-Square in a 2 × 2 table.

**Results**

All +AP and –AP changed significantly with time (Figs. 1 and 2). Retinol, albumin and cholesterol concentrations decreased and reached a nadir around calving (P < 0.001), and increased thereafter. The individual LAI, based on the concentration of these –AP, ranged from −0.94 to 0.98; the mean LAI was −0.51 ± 0.08 in Group 1 and 0.45 ± 0.12 in Group 2. Concentrations of PON were affected by time (P = 0.037) and tended to differ between groups (P = 0.078). Additionally, a time × group interaction was present (P = 0.056); a PON decrease around calving was noted in all cows, but appeared to be more pronounced in Group 1 than in Group 2.

Caeruloplasmin concentrations increased at calving (P = 0.002) and remained elevated throughout the study. Group 1 displayed higher caeruloplasmin levels than Group 2 (P = 0.036). A time effect on haptoglobin was found (P < 0.001); concentrations increased at calving and then recovered towards basal levels. Although average haptoglobin peaks appeared to be higher in Group 1, no group effect was found (P = 0.33).

There was an association between disease incidence and group (P = 0.024). In Group 1, 6/10 cows developed visible disorders during the first 6 weeks of lactation, including dystocia (n = 1), endometritis (n = 3) (as defined by Sheldon et al., 2006), mastitis (n = 1), and a combination of endometritis and abdominal displacement (n = 1). On average, the diagnosis was made on 10 days pp. In Group 2, one cow developed fever, anorexia, and milk drop on 21 days pp.

The change with time after calving in the liver variables is shown in Fig. 3. Bilirubin was affected by time (P < 0.001) but not by group (P = 0.54). There was an interaction between time and group (P = 0.001); in Group 2, bilirubin increased around calving and remained elevated, while the increase in Group 1 tended to be higher and was followed by a progressive decrease. Similarly, for AST there was an effect of time (P < 0.001) but not of group

![Fig. 1. The concentration of positive acute phase reactants (A) caeruloplasmin; (B) haptoglobin) on several time points relative to parturition in high-yielding dairy cows. Cows were classified according to their liver activity index; Group 1 (dark bars; n = 10) had a lower value of index, suggestive of more pronounced inflammation, compared to Group 2 (light bars; n = 11). Error bars represent the standard error of the mean.](image-url)
The interaction was significant at the 10% level \((P = 0.09)\) with higher peak AST activities being seen in Group 1 during the first week pp. GGT was unaffected by time \((P = 0.24)\) or group \((P = 0.15)\). An interaction was seen \((P = 0.008)\); in contrast to Group 2, cows in Group 1 displayed a clear postpartum increase in GGT.

**Discussion**

In this study, we aimed to depict and quantify the APR in early lactation dairy cows under field conditions based on +AP and −AP, and to assess the association of APR with disease incidence and liver variables.

Although associations between caeruloplasmin and haptoglobin concentrations and inflammatory events have been described in cattle, concentrations of these compounds are subject to considerable fluctuations between and within cows \((\text{Hirvonen et al., 1999; Chan et al., 2003; Nyman et al., 2008})\). In the current study, fluctuations in −AP concentrations were substantially lower than for +AP \((\text{see Figs. 1 and 2})\). Therefore, for a quantitative estimation of the inflammatory load, the LAI was proposed \((\text{Bertoni et al., 2008})\), combining the concentrations of three different −AP at three different time points rather than a single measurement. Decreases in cholesterol, apolipoprotein B-100 and RBP are generally known to be associated with inflammatory events \((\text{Oikawa and Katoh, 1997; Gruys et al., 2005; Bionaz et al., 2007; Bertoni et al., 2008})\). The confounding of −AP concentrations by alterations in feed intake \((\text{Wolf, 1984; Duske et al., 2009})\) is expected to be minimal in the current study, as all cows received the same diet and displayed similar milk yields and metabolite concentrations.
(Bossaert et al., 2008). Based on this analysis we suggest that in this study the categorization of the cows based on LAI primarily reflected differences in their hepatic – AP synthesis, and that LAI is a suitable estimator of inflammatory load.

Cows were retrospectively categorized based on their LAI. An association between group and disease incidence was observed, with disease incidence being notably higher in Group 1 than in Group 2 cows. Hence, the typical changes in +AP and – AP had a clear cause in some, but not all cows. The association between LAI and disease incidence further strengthens the biological relevance of LAI as an estimator of APR.

The list of APRs analyzed in this study is far from complete (Petersen et al., 2004). The study of other –AP and +AP, such as serum amyloid-A, in a larger number of cows displaying a wider disease spectrum may lead to a better understanding of their association with clinical and subclinical inflammation processes. Paraoxonase, an enzyme synthesized in the liver, is involved in the prevention of lipoprotein oxidation in man (Feingold et al., 1998). In cows, similar anti-oxidative functions have recently been described (Turk et al., 2008) but the biological function of PON remains largely unknown. In rodents, cytokine challenge reduces PON blood levels and hepatic mRNA abundance, indicating that PON responds to inflammation as a –AP (Feingold et al., 1998). The current study supports this hypothesis: a decrease of PON concentrations was noted in the early pp period, and was more pronounced in Group 1 than Group 2 cows.

Activities/concentrations of AST, GGT and bilirubin corresponded with previous reports (Bionaz et al., 2007; Bertoni et al., 2008). Interestingly, group differences in these variables and their close correlation with +AP and – AP (results not shown) may lead to the assumption that a pronounced APR alters hepatic bilirubin clearance and inflicts liver damage (Carlson, 2008; Hoffmann and Solter, 2008). However, the reported associations are purely observational, and liver parameters may be confounded by numerous external factors (Carlson, 2008). Therefore, the current dataset does not prove any causal relationships and should be interpreted with caution.

Conclusions

These data contribute to a better understanding of the transition period biology. Irrespective of group and disease incidence, typical increases in +AP and decreases in – AP were observed in all cows, identifying early lactation as a period of increased inflammatory load. A lower LAI was accompanied by a more pronounced +AP rise and a higher disease incidence, suggesting that LAI is a suitable estimate of APR. The observed difference in liver variables between cows with pronounced or mild declines –AP raises interesting questions about the association between APR and liver function, but requires further research.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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