Evaluation of different acute-phase proteins for herd health diagnostics in early postpartum Holstein Friesian dairy cows

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Abstract

This research communication describes (1) the comparison of acute-phase protein (APP) concentrations in transition dairy cows on different farms using both pooled and individual blood samples, and (2) the association among different APP and clinical health parameters. The first hypothesis was that early postpartum dairy cows from different farms differ in the level of inflammation, which might be determined using APP assays in both pooled and individual blood samples. The second hypothesis was that the APP haptoglobin (Hp) might be the most sensitive parameter to detect cows at risk of excessive postpartum inflammation concomitant with systemic disease. Serum concentrations of Hp, serum amyloid-A (SAA), total protein (TP), albumin (Alb), coeruloplasmin (Cp) and C-reactive protein (CRP) in 100 fresh lactating cows (within 0–8 d postpartum) from 10 farms were compared and associated to clinical health parameters (rectal body temperature, vaginal discharge (Metricheck™ score), rumen fill, vulvovaginal laceration) using both pooled and individual blood samples. Mean serum concentrations of Hp, SAA and TP revealed significant differences among farms. Pooled serum samples of farms showed high correlations with the mean of individual samples. Only Hp was significantly positively correlated to both body temperature and Metricheck™ score. In conclusion, Hp differentiates dairy farms regarding the inflammatory state of transition cows using individual and pooled serum samples within the first week postpartum. It also mirrors the individual degree of inflammation, thus proving to be a diagnostic parameter of high interest during the periparturient period.

The process of parturition in dairy cows invokes different risk factors such as trauma, stress, pain and infection, triggering an acute-phase response of the innate immune system. During this response, interleukins (IL), especially IL-1β, IL-6 and tumor-necrosis-factor-α, are released by macrophages and neutrophils at the site of injury promoting the synthesis of positive acute-phase proteins (APP) in the liver (Baumann and Gauldie, 1994). Those proteins accumulate in blood and fulfill specific tasks when it comes to the elimination of the noxious stimuli. Due to an increased production of positive APP in the liver, the biosynthesis of liver-derived plasma proteins such as albumin (Alb) and retinol-binding-protein (RBP) decreases during an APR. The latter proteins are, therefore, considered negative APP. In bovine pathology today, the most frequently used APP are haptoglobin (Hp) and serum-amyloid-A (SAA: Ceciliani et al., 2012).

Several studies have shown the ability of Hp and SAA to indicate the presence of subclinical and clinical disease in cattle, such as mastitis (Safi et al., 2009), metritis (Huzzey et al., 2009) and others. However, clinically healthy animals exposed to stress, due to regrouping for example (Giannetto et al., 2011), also show increased serum concentrations of both Hp and SAA.

Although the innate immune system plays an important role in regulating inflammatory processes, excessive inflammation during the periparturient period is known to occur and to impair recovery instead of enhancing it (Sordillo et al., 2009). Health conditions and inflammatory processes during the periparturient period of dairy cows have a detrimental impact on milk yield, milk composition and reproductive performance in the following lactation (Huzzey et al., 2015). Therefore, the early detection of cows at risk for excessive inflammatory reactions postpartum would be beneficial, since it would allow appropriate monitoring and treatment of affected animals. Besides monitoring of individual animals, it would additionally be helpful for farmers and veterinarians to be able to investigate the inflammatory state of the herd or specific groups of animals (e.g. transition cows). This study was conducted in order to (1) compare acute-phase protein (APP) concentrations in transition dairy cows on different farms using both pooled and individual blood samples, and (2) assess the
relationship, if any, between different APP and clinical health parameters. The first hypothesis was that early postpartum dairy cows from different farms would show different levels of inflammation and that this level might be determined using APP assays in both pooled and individual blood samples. The second hypothesis was that the APP haptoglobin (Hp) might be the most sensitive parameter to detect cows at risk of excessive postpartum inflammation concomitant with systemic disease.

Materials and methods
The experimental procedures reported herein were conducted with the approval of the Federal State Office of Occupational Safety, Consumer Protection and Health (animal care protocol number: 2347-A-3-1-2018).

Animals and farms
A total of 100 Holstein Friesian dairy cows from 10 different commercial dairy farms (size ranging from 1074 to 2638 cows per farm) were included in the study. Farms were selected based on their herd size (≥ 1000 cows), geographic location (within the northeast of Germany), housing and feeding (free stall, total mixed ration), and consistent recording of animal health, fertility and milking traits. From each farm, 10 cows within 8 d postpartum were selected for examinations and sampling based on their current health state and recent calving history: only cows that had experienced a light calving process (no stillbirth, twin birth or dystocia, no assistance at calving) and that did not show any signs of disease at first examination were enrolled in the study. Cows were excluded from the study if they were unable to stand or walk, showed high fever (>40°C) or retained fetal membranes.

Clinical examinations
Each cow was clinically examined for general health determining rumen fill on a 1–5 scale (reference shown in Supplementary Table S3) and rectal body temperature (digital thermometer VT 1831 Veterinary Thermometer, Klöfert AG, Germany). Vulvovaginal laceration was scored on a 0–2 scale (score 0 = no laceration, 1 = laceration <2 cm at the dorsal commissure of the vulva or lateral walls of the vulva/vagina, 2 = laceration ≥2 cm at the dorsal commissure of the vulva or at the lateral walls of the vulva/vagina, or both; reference shown in Supplementary Table S3). The Metricheck™ device (Simcrot Tech Ltd., Hamilton, New Zealand) (McDougal et al., 2007) was subsequently used to assess the character of vaginal discharge on a 0–3 scale (score 0 = clear and mucoid discharge, score 1 = mucopurulent discharge with <50% of pus, score 2 = mucopurulent discharge with >50% of pus, score 3 = brownish-reddish, watery and putrid smelling discharge).

Sampling
From each cow, one blood sample was collected from the coccygeal vessel into a plain tube (SARSTEDT AG & Co. KG., Germany). The samples were centrifuged for 10 min at 3500 g and the serum was subsequently divided into individual aliquots. In addition to individual cow samples, three pooled samples were prepared for each farm by pipetting equal volumes (1 ml) of the 10 individual samples into a common pooling tube. Samples were stored at −24°C until analysis. One sample was excluded due to visible hemolysis, resulting in a total of 99 individual samples and a total sample number of 129 (including 30 pooled samples).

APP measurements
Serum concentrations of Hp, TP, Alb and Cp were determined using colorimetric measurement methods on a chemical autoanalyzer (CA) (Cobas 8.000 C 701 Autoanalyzer, Roche Diagnostics, Switzerland). The mean intra and inter assay CV were 1.2% and 5.3%, 1.8% and 2.2%, 0.5% and 1.1%, and 1.1% and 2.1% for Hp, TP, Alb, and Cp, respectively. SAA and CRP were measured by a commercially available ELISA test kit (Tridelta Development, Ireland) according to the manufacturer’s instructions and an inhouse ELISA of the laboratory, respectively. The intra and inter assay CV were 8.4% and 12.0%, and <10%, respectively.

Statistical analysis
All data were analyzed using IBM SPSS Statistics version 25 for Windows. Descriptive statistics were performed aiming at simply ranking farms depending on APP concentrations in pooled and individual samples. A Kruskal–Wallis test was performed to test for significant differences among farms regarding sampling day, APP concentrations and rectal body temperature. A chi-squared test was used to test for significant differences among farms regarding the distribution of lactation number, Metricheck™ score, rumen fill score and vulvovaginal laceration score. Correlations and one-way analyses of variance (ANOVA) were performed to assess associations among APP and clinical health parameters. Pearson’s correlation coefficient or Spearman’s rank correlation coefficient was determined for equally (two continuous variables) and not equally (one continuous and one ordinal variable) scaled data pairs, respectively. Correlations were considered significantly different from zero if $P < 0.05$.

Results
Descriptive Statistics as well as Correlations among different APP are presented in the online Supplementary File including Supplementary Tables S1 and S2. Overall, mean sampling time point was 4.08 ± 1.99 d in milk (DIM) and mean lactation number was 2.39 ± 1.99, both parameters were not different among farms ($P > 0.05$).

Differentiation and ranking of farms by APP concentrations
Serum Hp and SAA concentrations revealed highly significant differences among farms ($P < 0.01$) whilst TP concentration also differed ($P < 0.05$, Table 1). The highest arithmetic means for Hp, SAA and CRP were found on farm 6 whilst farms 1, 2, 3 and 7 showed the lowest means for Hp. When a threshold of 0.65 g/L serum Hp was implemented (threshold value for non-metritic cows on day 3 p.p. according to Chan et al., 2010), means for these four farms remained below this threshold, whereas means of all other farms showed elevated serum Hp concentrations (Supplementary Fig. S1).

Correlation between pooled and individual samples
Hp, Cp and Alb concentrations measured in pooled serum samples of each farm showed highly significant correlations...
with the arithmetic mean of the individual samples (Table 1). The highest correlation was found between Hp concentrations in pooled serum samples and its mean concentrations measured in individual serum samples ($r_p = 0.99$).

### Association between APP and health parameters

Only Hp showed significant correlations with both the Metricheck™ score ($r = 0.48$, $P_s < 0.001$) and rectal body temperature ($r_p = 0.45$, $P_p < 0.001$) (Fig. 1a and b, and Supplementary Table S3). Alb concentrations showed a significant negative correlation with the Metricheck™ score ($r = -0.40$, $P_s < 0.001$) (Fig. 1c). TP was negatively correlated with rectal body temperature ($r_p = -0.26$, $P_p = 0.009$) (Fig. 1e). No correlations were found between any APP and vulvovaginal laceration or rumen fill (Supplementary Table S3).

### Discussion

**Differentiation and ranking of farms by APP concentrations**

In this study, only SAA and Hp concentrations revealed highly significant differences among farms. This might point to their sensitivity for farm-specific inflammatory reactions provoked by,
on the one hand, environmental stressors such as regrouping (Giannetto et al., 2011) and, on the other, farm-specific diseases, such as mastitis (Safi et al., 2009), and metritis (Huzzey et al., 2009). In addition, this finding corresponds to the fact that from all of the APP tested in this study, Hp and SAA are those most frequently used to detect inflammation in dairy cows (Ceciliani et al., 2012). However, the fact that only SAA and Hp revealed highly significant differences among farms might also be attributable to the sampling time frame (within 0–8 DIM), since both proteins are known to be so called major and first-line APP, reacting more intensely and faster to proinflammatory stimuli compared to other APP (Ceciliani et al., 2012). Considering the strong correlation in Hp concentration between individual and pooled serum samples found in this study ($r = 0.992$), there might be a potential value for analyzing Hp to determine the inflammatory status of farms using pooled serum samples from selected animals within the first week postpartum.

**Correlation among pooled and individual samples**

In general, pooled samples of each farm showed high correlations with individual samples (Table 1), underlining their suitability for herd-level analyses of inflammatory reactions in transition dairy cows.

**Association between APP and clinical health parameters**

From all APP tested in this study, Hp showed the strongest correlation to the Metricheck™ score and rectal body temperature. The Metricheck™ device, developed by McDougall et al. (2007), allows an easy and clean observation of vaginal discharge, hence supporting the diagnosis of uterine disease in cows. Previous studies have shown that serum Hp is significantly elevated in cows with metritis and that its concentration increases along with the severity of this disease (Huzzey et al., 2009). In contrast to the present study, Kaya et al. (2016) reported that Cp and SAA concentrations also increase significantly and correlate well with the severity of endometritis. A possible explanation for these discrepancies might be the time of the examination. In the present study, examinations were performed within 0–8 d p.p. whereas Kaya et al. (2016) examined the cows on day 28–32 p.p. Hp might be more sensitive in cases of uterine disease and thus react earlier than SAA and Cp. Our results are also in agreement with the findings of Brodzki et al. (2015). These

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**Fig. 1.** Boxplots (a, c, e) and scatterplots (b, d, f) illustrating the association between the concentrations of haptoglobin (a, b), albumin (c, d), and total protein (e, f) in serum of cows within 0–8 d p.p. with the Metricheck™ score and rectal body temperature, respectively.
authors reported elevated Hp values already on day 5 postpartum in cows that later developed subclinical endometritis, whereas SAA values did not increase before day 22 postpartum.

We found serum Hp concentrations to correlate with rectal body temperature. This could be explained by the increase in body temperature in several cases of inflammatory processes concomitant with the release of interleukins, primarily IL-6, and thus with the production of APP in the liver. However, none of the other APP showed this association to body temperature, which could be attributed to the time of examination. Additionally, the colorimetric Hp assay used in this study was more precise than the SAA-ELISA (intra and inter assay CV of 1.2% and 5.3%, and 8.4% and 12.0%, respectively) which might further explain our findings.

In conclusion, the present study underlines the potential application of Hp for an early diagnosis of excessive inflammatory reactions in the periparturient period of dairy cows at both the farm and individual level. Pooled samples might be useful for status analyses of farms regarding inflammatory processes in fresh lactating dairy cows, but further research is needed to establish reference values for this purpose. At the individual level, Hp was the most sensitive diagnostic marker for early postpartum uterine disease and general inflammatory reactions.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029921000078

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