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Associations between serum haptoglobin concentration and peri- and postpartum disorders, milk yield, and reproductive performance in dairy cows

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ABSTRACT

We determined the associations between serum haptoglobin concentration and peri- and postpartum disorders, milk yield, and reproductive performance in dairy cows. Furthermore, associations between haptoglobin and some blood metabolites were also investigated. We collected blood from 112 Holstein cows 1 week postpartum to measure serum haptoglobin and metabolite/enzyme (aspartate aminotransferase [AST], total bilirubin [T-bilirubin], total cholesterol [TCH], non-esterified fatty acids [NEFA], and β-hydroxybutyrate [BHBA]) concentrations. Cows were divided into two groups based on their haptoglobin concentrations: a low-HP group (<100 μg/mL, n = 72) and a high-HP group (>100 μg/mL, n = 40). The incidence of peri- and postpartum disorders (dystocia, retained placenta, metritis, and endometritis) was higher (P < 0.05–0.01) in the high-HP group than in the low-HP group, whereas milk yield during the first 2 months postpartum was lower (P < 0.05–0.01) in the high-HP group than in the low-HP group. Moreover, the probability of conception by 210 days postpartum was also lower (hazard ratio: 0.55; P < 0.05) in the high-HP group than in the low-HP group. Serum AST, T-bilirubin, NEFA, and BHBA concentrations were higher in the high-HP group than in the low-HP group (P < 0.05–0.01), whereas TCH concentration was lower (P < 0.01) in the high-HP group than in the low-HP group. In conclusion, our results indicate that high serum haptoglobin concentration 1 week postpartum was associated with a greater incidence of peri- and postpartum disorders, and reductions in milk yield and reproductive performance, in dairy cows. It was also associated with high serum AST, T-bilirubin, NEFA, and BHBA concentrations, but low TCH concentration.

1. Introduction

During their transition period, dairy cows experience metabolic stress due to negative energy balance (NEB), which leads to reduced liver function and immune responses, resulting in a higher incidence of several postpartum diseases, lower productivity and, impaired reproductive performance (Bertoni et al., 2008; Bionaz et al., 2007; Hammon et al., 2006). Earlier detection of postpartum metabolic, inflammatory, immune, and postpartum complications using a biomarker might help to prevent economic loss resulting from the expense of disease treatment, culling, lower productivity, and impaired reproductive performance. Therefore, several blood metabolites and enzymes, including non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), and aspartate aminotransferase (AST), were evaluated for their usefulness as predictors of postpartum health, and the subsequent productivity and reproductive performance of dairy cows (González et al., 2011; Huzzey et al., 2011; Ospina et al., 2010; Qu et al., 2014).

Haptoglobin, an acute phase protein, is released by hepatocytes during inflammatory conditions in response to proinflammatory cytokine production (Ametaj et al., 2005). Thus, serum haptoglobin concentration reflects the severity of inflammatory responses; its normal serum concentration is negligible in healthy animals, but increases over 100-fold after immune stimulation in ruminants (Eckersall and Bell, 2010; Kaya et al., 2016). Previous studies report that haptoglobin could be a useful diagnostic and prognostic marker for several diseases, including mastitis, enteritis, peritonitis, pneumonia, endocarditis, and endometritis, in cattle (Murata et al., 2004; Petersen et al., 2004). In addition, high serum haptoglobin concentration has been associated with calving difficulties, retained placenta, and metritis in dairy herds (Huzzey et al., 2009; Pohl et al., 2015). Interestingly, a few publications show that high plasma haptoglobin concentration during the early

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postpartum period is associated with a subsequent decrease in reproductive performance in dairy cows (Chan et al., 2010; Huzzey et al., 2015; Nightingale et al., 2015). Despite numerous studies of the use of haptoglobin as a marker of inflammatory diseases, objective standardized values have not been defined. Moreover, investigations of the associations between haptoglobin concentration and milk yield and reproductive performance have been limited to date (Huzzey et al., 2015).

A few publications report relationships between haptoglobin and other blood markers (NEFA, BHBA, or AST) in postpartum dairy cows (Galvão et al., 2010; Kaya et al., 2016; Pohl et al., 2015), which may indirectly indicate nutritional, inflammatory, and immune status during the early postpartum period. Moreover, relationships among haptoglobin, blood metabolites, peripartum disorders, and productivity and reproductive outcome have not been clearly determined. However, if serum haptoglobin represents a useful predictor of peri- and postpartum disorders, productivity, and reproductive performance, it could contribute to the prevention of economic loss in the dairy industry. Therefore, the objective of this study was to determine the associations between serum haptoglobin concentration 1 week postpartum and blood metabolites/enzymes (AST, total bilirubin [T-bilirubin], total cholesterol [TCH], NEFA, and BHBA), peri- and postpartum disorders, milk yield, and reproductive performance in dairy cows.

2. Materials and methods

2.1. Animals

This study was performed on four Holstein dairy farms, located in Chungcheong Province. The cows were maintained in loose housing systems, fed total mixed rations, and milked twice daily. A total of 112 Holstein dairy cows (24 primiparous and 88 multiparous) were included in the study. Milk yield was measured every day for each cow during the first 2 months postpartum. All experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Korea.

2.2. Blood sampling and study design

Blood was collected from the tail vein of the cows 1 week (± 1.1 days) postpartum to measure serum haptoglobin and metabolite/enzyme concentrations. Cows were divided into two groups based on their serum haptoglobin concentrations: a low-HP group (< 100 μg/mL; n = 72) and a high-HP group (> 100 μg/mL; n = 40). Associations between serum haptoglobin concentration (low or high) and serum metabolites/enzymes (AST, T-bilirubin, TCH, NEFA, and BHBA), peri- and postpartum disorders (dystocia, retained placenta, metritis, and endometritis), milk yield during the first 2 months, and reproductive performance (probability of conception by 210 days postpartum) were determined.

Ten milliliters of blood was collected from each cow into plain plastic centrifuge tubes, which were immediately placed on ice. The samples were then centrifuged at 2000 × g for 10 min at 4 °C, and the serum was harvested and frozen at −80 °C until assayed.

2.3. Case definitions and reproductive management

The definitions of the peri- and postpartum disorders that were used in the present study were similar to those described previously (Cook et al., 2006; López de Maturana et al., 2007; Sheldon et al., 2006). Calving difficulty was ranked according to the degree of assistance required (1 = no assistance, 2 = minor assistance, 3 = some force required, 4 = significant force required, and 5 = caesarean section). Cows with a calving difficulty score > 2 were considered to have dystocia. Retained placenta was defined as the retention of the fetal membranes for longer than 24 h. Metritis was defined by the presence of fever (≥ 39.5 °C) and a watery, fetid uterine discharge during the first 10 days postpartum. Endometritis was diagnosed at week 4 postpartum by examining the vaginal discharge using the Metricheck tool (McDougall et al., 2007); cows with a mucopurulent discharge (< 50% pus) were diagnosed with endometritis.

The voluntary waiting period from calving to the first artificial insemination was 40 days. Pregnancy was diagnosed 35–40 days after insemination by transrectal ultrasonography. Data on reproductive performance were collected for a minimum of 210 days postpartum or until pregnancy or culling.

2.4. Measurement of serum haptoglobin and metabolite/enzyme concentrations

Haptoglobin concentration was determined using a commercially available bovine haptoglobin ELISA test kit (Life Diagnostics, Inc., West Chester, PA, USA). All procedures were performed according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were 3.1% and 6.7%, respectively.

The concentrations of AST, T-bilirubin, TCH, NEFA, and BHBA in serum samples were measured using a 7180 Biochemistry Automatic Analyzer 710 (Hitachi Ltd., Tokyo, Japan) and commercial enzyme assay kits (Wako Pure Chemical Ltd., Osaka, Japan), according to the guidelines provided by the manufacturer. The intra- and inter-assay coefficients of variation were < 5% for all these assays.

2.5. Statistical analyses

Data are expressed as mean ± standard error of the mean (SEM). For statistical analyses, cows were defined as either primiparous or multiparous, and the calving season was defined as spring (March to May), summer (June to August), autumn (September to November), or winter (December to February). Statistical analyses were performed using SAS software (version 9.4, SAS Inst., Cary, NC, USA).

Serum haptoglobin concentration was compared between primiparous and multiparous cows using Student's t-test. Haptoglobin concentration was not normally distributed; therefore, values were transformed to their natural logarithms for data analysis, although untransformed data (mean ± SEM) are presented herein. The incidence of peri- and postpartum disorders (dystocia, retained placenta, metritis, and endometritis) was compared between the groups using chi-square or Fisher's exact tests.

Milk yield was analyzed using mixed models. The statistical models included group (low-HP and high-HP), time of measurement (1 and 2 months postpartum), and two-way interactions between group, cow parity, and measuring time. Student's t-test was performed when a group effect was observed.

Cox's proportional hazard model with the PHREG procedure was used to analyze the probability of conception by 210 days postpartum among the groups. This estimated the chance of a cow being pregnant at a given time. The time variables used in this model were the interval in days between calving and pregnancy. Cows that died, were sold, or were not pregnant by 210 days postpartum were not included in the analysis. Cox models included farm identity, calving season, cow parity (primiparous or multiparous), and group (low-HP and high-HP). The proportional hazard rate was determined based on interactions between explanatory variables and time, and by evaluating Kaplan–Meier curves. The median and mean days between calving and pregnancy were determined by survival analysis using the Kaplan-Meier model and the LIFETEST procedure within the SAS software. A survival plot was generated using the survival option within MedCalc software (11.1, MedCalc Software, Mariakerke, Belgium).

Statistical analysis of serum metabolites/enzymes (AST, T-bilirubin, TCH, NEFA, and BHBA) was carried out using the general linear models procedure. This model included cow parity, calving season, and group (low-HP and high-HP). $P < 0.05$ was considered significant.