

Serum Haptoglobin as an Indicator for Calving Difficulties and Postpartal Diseases in Transition Dairy Cows

By

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Abstract

Haptoglobin is an acute phase protein that is elevated in response to tissue damage and infections in dairy cows. The objectives of this study were to evaluate whether serum haptoglobin concentrations in the first week after calving can indicate disease status and severity, and whether haptoglobin concentrations are elevated before clinical signs of disorders or diseases are detected. Blood samples were taken from 161 Holstein cows (2 to 7 parities) at days -21 (-26 to -18), -14 (-17 to -11), -7 (-10 to -5), -3 (-4 or -3), -1 (-2 or -1), 0, 1, 3, 7, 14, 21, and 28 postpartum and analyzed for serum haptoglobin concentrations. Between days -21 and 100 postpartum, cows were monitored for signs of diseases and treated according to standard treatment protocols. Based on disease status and severity, cows were grouped into healthy cows ($n = 20$), cows with mild or subclinical disease ($n = 41$), cows with severe disease requiring antibiotics, glucose precursors, or both ($n = 70$), and cows that died or were sold within the first 100 d postpartum ($n = 30$). Haptoglobin areas and peak concentrations in the first week after calving were greater in sick versus healthy cows ($P < 0.003$) and in severely sick cows versus cows with mild or subclinical diseases ($P < 0.001$). Postpartal peak area and concentrations also differed or tended to differ between number and type of diseases (ketosis only, metritis only, mastitis only, other diseases only, 2 or more diseases). Based on the first treatment time, cows were grouped into not-treated cows ($n = 40$), cows first treated day 8 to 28 postpartum ($n = 36$), cows first treated day 4 to 7 postpartum ($n = 32$), cows first treated day 0 to 3 postpartum ($n = 39$), and cows first treated prepartum ($n = 14$). Prior to first treatment, sick cows had greater haptoglobin concentrations than not-treated cows (all $P < 0.01$). Cows with birth complications (twins, dystocia) had elevated haptoglobin concentrations two weeks before calving ($P < 0.001$).

Our results suggest haptoglobin area and peak concentrations in the first week after calving can indicate disease status and severity and that elevated haptoglobin concentrations can precede birth complications and clinical onset of diseases during early lactation. Thus, serum haptoglobin concentrations may assist in early detection and treatment of sick cows.

Key Words: dairy cows, disease, haptoglobin, indicator.

Introduction

The transition period in the dairy cow can be defined as the change from a gestational non-lactating to a non gestational lactating state. The period, which spans from 3 weeks before through 3 weeks after parturition, is associated with an elevated incidence of metabolic and infectious diseases (LeBlanc et al., 2010). A major reason for the increased disease risk is that cows are exposed around calving to metabolic and infectious challenges, including limited appetite combined with greater nutrient demands, a switch from low- to high-concentrate diet, and increased exposure and susceptibility of the mammary gland and uterine tract to bacteria.

Haptoglobin is an acute phase protein that is elevated in response to infections and tissue damage, including parturition, in dairy cows (Humblet et al., 2006). Haptoglobin, primarily synthesized in the liver, usually increases one day after the original challenge and remains elevated for up to two weeks (Jacobson et al., 2004; Vels et al., 2008). The haptoglobin response is proportional to the severity of the challenge (Jacobson et al., 2004; Vels et al., 2008). The time course and extent of haptoglobin response is in concordance with the primary role of haptoglobin to prevent further tissue damage and promote tissue repair (Peterson et al., 2004; Ametaj et al., 2011). Haptoglobin accomplishes its function by acting as an antioxidant, anti-inflammatory agent, and tissue-regeneration agent, bacteriostat, and by regulating the maturation and activity of immune cells. Thus, haptoglobin has been proposed as an indicator of acute and chronic diseases; however, the sensitivity of haptoglobin for disease detection is limited (Horadagoda et al., 1999; Humblet et al., 2006). Reasons for the limited sensitivity of haptoglobin are that not all cows have elevated haptoglobin concentrations during disease, haptoglobin concentrations decrease after an acute infection, and the haptoglobin response to

tissue damage or infection is delayed (Horadagoda et al., 2004; Jacobson et al., 2004; Vels et al., 2008).

Few studies examined the efficacy of haptoglobin concentrations for early detection of disease. Huzzey et al. (2009) reported elevated serum haptoglobin concentrations in the first days postpartum precede the clinical onset of metritis. Similarly, Dubuc et al. (2010) reported elevated serum haptoglobin concentrations during the first week after calving are associated with a greater incidence of clinical metritis. Prepartal haptoglobin concentrations were, however, not significantly associated with any or severe disease during the first 30 days of lactation (Huzzey et al., 2011).

The first objective of the current study was to evaluate whether peripartal haptoglobin concentrations are associated with health status and disease severity during the first 4 weeks postpartum, and type and number of diseases. The second objective was to examine whether prepartal haptoglobin concentrations can indicate birth complications or clinical disease immediately after calving, and whether postpartal haptoglobin concentrations are elevated before clinical signs of diseases were detected. Our hypothesis was that elevated peripartal haptoglobin concentrations are associated with disease incidence and severity, and type and number of diseases during the first 4 weeks postpartum and that elevated haptoglobin concentrations precede clinical signs of disease

Materials and Methods

All procedures involving animals were conducted in accordance with Oregon State University Institutional Animal Care and Use (ACUP No. 3991).

Animal Management

The research was conducted on a 1,000-head commercial dairy farm in Oregon's Central Willamette Valley during Spring and Summer 2010. A total of 161 multiparous Holstein cows (parity 2 to 7) participated in the study, which lasted from four weeks before predicted calving date to 28 days postpartum. Prepartum, the cows were housed in a group of 40 to 50 cows in a straw-bedded free stall barn. Cows were fed once in the morning (7:30) a typical dry-cow total mixed ration (TMR), which was formulated according to NRC guideline (NRC, 2001).

After calving, cows were moved to the hospital pen that housed 30 to 40 cows. The hospital pen was used for fresh cows (first two days postpartum) and cows needing major medical treatment including antibiotic treatment requiring the milk to be discarded, oral or intravenous treatment with energy precursors, or both. Cows from the hospital pen were fed twice a day (7:00 and 12:30) a typical fresh cow TMR, which was formulated according to NRC guidelines (NRC, 2001). Cows from the hospital pen were milked twice a day at 6:00 and 16:00. The other cows in the first month of lactation were grouped in an early lactation pen that housed 90 to 110 cows. Cows from the early lactation pen were fed twice a day (9:00 and 13:00) the same TMR as the cows in the hospital pen. Cows from the early lactation pen were milked daily four (for the first three months of the study) and six times (for the remaining three months of the study) at 8:45 and then every 6 or 4 hours, respectively. After the first month of lactation, cows were grouped based on body size in three mid-lactation pens that housed 90 to 110 cows each. Cows in the mid-lactation pens were fed twice a day (10:00 and 13:30) the same TMR as the

cows in the early lactation pen. Cows from the mid-lactation pen were milked daily three times at 10:00 and then every 8 hours, respectively.

During the peripartal period, three evaluators independently determined once weekly body condition scores (BCS) on a five-point scale (Edmonson et al., 1989). Because the commercial dairy had no automatic milk meters, a certified milk tester from Willamette Valley DHIA (Salem, OR) measured milk production twice weekly on non-consecutive days during the mid-afternoon milking. Milk samples were analyzed for fat, protein, lactose, and somatic cell concentrations at Willamette Valley DHIA using mid-infrared spectrophotometry. Between day -28 and 100 postpartum, cows were monitored daily for signs of diseases. Medical treatments were administered and recorded by the herd manager and categorized by disease in Dairy Comp (Valley Ag. Software, Inc., Tulare, CA). Any medical treatments were administered based on a standard operating procedure of the dairy farm.

Blood Collection and Analysis

Blood samples were taken from at day -21 (-26 to -18), -14 (-17 to -11), -7 (-10 to -5), -3 (-4 or -3), -1 (-2 or -1), 0, 1, 3, 7, 14, 21, and 28 postpartum. Within 10 minutes after morning lockup and feeding, 5 to 8 mL of blood were obtained from the coccygeal vein or artery in 10 mL serum vacutainer tubes (BD Vacutainer[®] Plus Plastic Serum Tubes, BD Diagnostics, Franklin Lakes, NJ). Blood samples were placed on ice immediately after collection. Blood samples were transported on ice to the laboratory and serum was separated by centrifugation at room temperature for 20 minutes at 1600 x g. Serum samples were stored at -20°C until chemical analysis. Serum haptoglobin concentrations were measured using a bovine-specific ELISA kit (Catalog No. 2410-70; Life Diagnostics, Inc., West Chester, PA) and serum β -hydroxybutyrate (BHBA) concentrations were measured using an enzymatic assay (Stanbio

BHBA LiquiColor[®] Procedure No. 2440; Stanbio Laboratory) according to manufacturer's instructions, using a FLUOstar Omega microplateautoreader (BMG Labtech Inc, San Francisco, CA). The intra-assay and inter-assay CV for haptoglobin was 3.0 and 14.6%, respectively.

Classification of Groups

To evaluate the association between peripartal serum haptoglobin concentrations and disease status and severity, cows were classified into four groups. Cows classified as being healthy cows (Healthy: n = 19) did not receive medical treatment, had a milk somatic cell concentrations below 1,000,000 cells/mL in milk, and had serum BHBA concentrations below 1.3 mmol/L during the study period (day -21 to 28 postpartum). Cows classified as having mild disease (Mild Disease: n = 49) were treated with medications without withdrawal period that did not include glucose precursors, or had at least for part of the study period milk somatic cell concentrations above 1,000,000 cells/mL in milk or serum BHBA concentrations above 1.3 mmol/L. Cows, classified as having severe disease (Severe Disease: n = 63), were treated with antibiotics that required a withdrawal period for the produced milk, received orally or i.v. glucose precursors, or both. Cows classified as died or sold (Died/Sold: n = 30), died or were sold during the first 100 days postpartum. To evaluate the association between serum haptoglobin concentrations and disease status, the average of the three sick-cow groups (mild disease, severe disease, and died/sold) was compared versus the healthy group (healthy). To evaluate the association between serum haptoglobin concentrations and disease severity, the average of the two severe-sick cow groups (severe disease and died/sold) was compared to the mild-disease group (mild disease). In addition, the severe disease group was compared to the died/sold group.

To evaluate the association between serum haptoglobin concentrations and disease number and type, cows were classified into seven groups: (1) cows that were healthy during the study period (Healthy: n = 20), (2) cows with mild disease (Mild Disease: n = 17), (3) cows with diseases other than ketosis, metritis, or mastitis (Other Disease: n = 19), (4) cows with BHBA concentrations above 1.3 mMol/L during the study period but no other major diseases (Ketosis: n = 20), (5) cows with placental retention, mucopurulent, purulent, or putrid vaginal or cervical discharge, or both, but no other major disease (Metritis: n = 21), (6) cows with milk flakes, clinical mastitis (pain, swelling), or somatic cell concentrations above 1,000,000 cells/mL more than once but no other major disease (Mastitis: n = 17), and (7) cows with more than one disease (2+ Diseases; n = 47). To determine whether the number of diseases affected serum haptoglobin concentrations, the four groups with a single disease (other disease, ketosis, metritis, and mastitis) were compared to the 2+ disease group. To determine whether the association between serum haptoglobin concentrations and disease type differed, the four groups with a single disease were compared with each other using the three following orthogonal contrasts. To determine whether the haptoglobin response to ketosis, metritis, and mastitis differs from the response to other diseases, the other disease group was compared to the average of the ketosis, metritis, and mastitis groups. To determine whether cows with infectious and cows with metabolic diseases differ in serum haptoglobin concentrations, the ketosis group was compared to the average of the mastitis and metritis groups. To determine whether cows with mastitis and cows with metritis differ in serum haptoglobin concentrations, the metritis group was compared to the mastitis group.

To evaluate the association between serum haptoglobin concentrations and birth complications, cows were classified into 3 groups: (1) healthy cows and cows with mild disease

(Healthy/Mild Disease: n = 63), (2) cows with severe disease that did not have birth complications, including those that died or were sold with the first 100 days postpartum (Other Severe Disease: n = 70), and (3) cows with birth complications (Birth Complications; n = 28) such as twins (n = 16), requiring a hard pull or a C-section (n = 8), or both (n = 4). The group of cows with birth complications was compared to the healthy/mild disease group and the group average of the two groups with no birth complications (healthy/mild disease and other severe disease).

To evaluate the association between serum haptoglobin concentrations and first treatment time, cows were grouped into 5 groups: (1) cows that were not treated between day -21 and 28 postpartum (No Treatment: n = 39), (2) cows that were first treated between d 8 and 28 postpartum (Treated D8 to 28: n = 33), (3) cows that were first treated between d 4 and 7 postpartum (Treated D4 to 7: n = 25), (4) cows that were first treated between d 0 and 3 postpartum (Treated D0 to 3: n = 50), and (5) cows that were first treated between d -21 and -1 postpartum (Treated D-21 to -1: n = 14). The group of not treated cows was compared to each treated group.

Statistical Analysis

Data was analyzed as repeated-measures-in-time study using the PROC MIXED procedure of SAS (SAS Institute, 2001). To account for the unequally spaced repeated measures, the variance-covariance structure of repeated measures within cow was modeled using the first-order antedependence variance-covariance matrix, which was the most parsimonious variance-covariance matrix based on the Akaike Information Criterion. Fixed effects in all models included sampling time (day -21, -14, -7, -3, -1, 0, 1, 3, 7, 14, 21, and 28 postpartum), body condition at the beginning of the study (thin cow: BCS < 3.5; normal body condition: BCS

3.5 to 4.0; obese cow: BCS > 4), or parity (2, 3, and 4 or more), and the interactions between sampling time and body condition and between sampling time and parity. In addition, either disease status and severity (healthy, mild disease, severe disease, and sold/died), disease type (healthy, mild disease, other disease, ketosis, metritis, mastitis, and 2+ diseases), birth complications (healthy/mild disease, other severe disease, and birth complications), or first treatment time (Not treated, Treated D8 to 28, Treated D4 to 7, Treated D0 to 3, and Treated D-21 to -1) and their interaction with sampling time was included as fixed effects in the statistical model to test their respective association with serum haptoglobin concentrations.

To evaluate the haptoglobin response to the disease, the maximum concentrations and the area under the curve (AUC) were calculated for each of the following sampling time periods: prepartum (D-21 to 0; Prepartum), first week postpartum (D0 to 7; Postpartum Wk 1), and the remaining postpartum period (D7 to 28; Postpartum Wk 2-4). The maximum concentrations were used as indicator of peak response, and the AUC values were used as indicator of total response. To account for differences in the length of the sampling time periods, the AUC values were divided by the number of days of the respective sampling time period. The associations between peak and total haptoglobin response and disease status and severity, disease type, birth complications, or first treatment time were evaluated in PROC GLM using a model that included body condition (thin, normal, obese), parity (2, 3, and 4 or more parities), and their respective groups.

Values presented in the figures and tables are geometric least-squares means and their standard errors (SEM). All statistical tests were two-sided. Significance was declared at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

Results

Serum Haptoglobin is a Potential Indicator of Disease Status, Severity, Number, and Type

Serum haptoglobin concentrations were associated with incidence of disease in peripartal dairy cows. Cows with clinical or subclinical disease during the peripartal period had greater serum haptoglobin concentrations than healthy cows ($P < 0.001$; Figure 1). There was, however, a significant time by disease status interaction ($P < 0.001$), as the relationship between haptoglobin concentrations and health was most pronounced in the first week after calving ($P_{\text{Wk 1 pp}} < 0.001$; Figure 1). Compared to healthy cows, cows with clinical or subclinical disease had greater peak concentrations ($P < 0.001$; Figure 2) and AUC values ($P < 0.001$; Figure 3) in the first week after calving. In summary, peak and total haptoglobin response in the first week postpartum were indicators of health status of dairy cows in the peripartal period.

Serum haptoglobin concentrations were associated with disease severity in peripartal dairy cows. Cows of the two severe sick groups had greater serum haptoglobin concentrations than cows with mild or subclinical disease during the peripartal period ($P < 0.001$; Figure 1). There was, however, a significant time by disease severity interaction ($P < 0.001$), as the relationship between haptoglobin concentrations and disease severity was most pronounced in the first week after calving ($P_{\text{Wk 1 pp}} < 0.001$; Figure 1). Compared to cows with mild or subclinical disease, cows with severe diseases had greater peak concentrations ($P < 0.001$; Figure 2) and AUC values ($P < 0.001$; Figure 3) in the first week after calving. Compared to cows with severe disease, cows that died or were sold had greater peak concentrations ($P = 0.04$; Figure 2) and AUC values ($P = 0.02$; Figure 3) in week 2 to 4 after calving. Our results indicate that cows that died or were sold had chronically elevated haptoglobin concentrations in the peripartal

period. In summary, peak and total haptoglobin response in the first week postpartum were indicators of disease severity of dairy cows in the peripartal period.

The association between serum haptoglobin concentrations and peripartal diseases differed between number and type of diseases (Figures 4 and 5). Cows with two or more diseases had or tended to have significant higher haptoglobin concentrations in the first week postpartum ($P_{\text{Peak Response}} = 0.08$; $P_{\text{Total Response}} = 0.06$) and in weeks 2 to 4 postpartum ($P_{\text{Peak Response}} = 0.13$; $P_{\text{Total Response}} = 0.04$) than cows with one disease only. Cows with different types of diseases differed or tended to differ in haptoglobin concentrations in the first week postpartum ($P_{\text{Peak Response}} = 0.03$; $P_{\text{Total Response}} = 0.08$) and in weeks 2 to 4 postpartum ($P_{\text{Peak Response}} = 0.005$; $P_{\text{Total Response}} = 0.02$). The difference in haptoglobin concentrations between disease types in the first week was primarily the result of higher haptoglobin concentrations in cows with ketosis, metritis, and numerically for mastitis versus cows with other diseases ($P_{\text{Peak Response}} = 0.02$; $P_{\text{Total Response}} = 0.08$). The differences in haptoglobin concentrations between disease types in weeks 2 to 4 postpartum was primarily the result of higher haptoglobin concentrations in cows with mastitis versus cows with metritis ($P_{\text{Peak Response}} < 0.001$; $P_{\text{Total Response}} = 0.008$). In summary, peak and total haptoglobin concentrations during week 1 and week 2 to 4 after calving were indicators for number and type of diseases.

Elevated Serum Haptoglobin Precedes Birth Complications and Clinical Disease Diagnosis

Elevated serum haptoglobin concentrations were associated with birth complications (twins, hard pull, or both). Cows with birth complications did not only have greater haptoglobin concentrations in the first week postpartum ($P < 0.001$) compared to cows without birth complications, but also prepartum at days -14 ($P < 0.001$) and -7 ($P = 0.01$; Figure 6).

Compared to cows without birth complications, cows with birth complications had greater peak

concentrations ($P = 0.004$) and AUC values ($P < 0.001$) in the last 3 weeks prepartum (Figure 7). In summary, elevated serum haptoglobin concentrations preceded birth complications.

Serum haptoglobin concentrations were elevated a few days before clinical signs of the disease were detected and treated. Treated cows had greater haptoglobin concentrations for the last week before treatment than cows that were not treated in the peripartal period ($P_{D -7 \text{ to } -1 \text{ postpartum}} = 0.03$ for cows treated day 0 to 7 postpartum; $P_{D -3 \text{ to } 3 \text{ postpartum}} = 0.01$ for cows treated day 4 to 7 postpartum; $P_{D 0 \text{ to } 7 \text{ postpartum}} < 0.001$ for cows treated day 8 to 28 postpartum; Figures 8 to 10). Specifically, cows that were first treated within day 0 to 3 postpartum had greater haptoglobin concentrations at day -7 ($P = 0.05$) and -3 postpartum ($P = 0.01$; Figure 8). Cows that were first treated between 4 and 7 days postpartum had greater haptoglobin concentrations at day 1 ($P = 0.04$) and 3 postpartum ($P < 0.001$; Figure 9). Cows that were first treated between 4 and 7 days postpartum had greater haptoglobin concentrations at day 3 ($P = 0.002$) and 7 postpartum ($P < 0.001$; Figure 10). In summary, elevated serum haptoglobin concentrations preceded clinical disease diagnosis and treatment.

Discussion

The objective of this study was to evaluate serum haptoglobin concentrations as a potential indicator of birth difficulties and diseases in cows during the peripartal period. We observed that elevated serum haptoglobin concentrations during the first week postpartum indicated incidence, severity, number, and type of disease and that elevated serum haptoglobin concentrations preceded birth complications and clinical diagnosis and treatment of peripartal diseases. Our results suggest that inflammation precedes the clinical onset of peripartal diseases.

Inflammation has been previously proposed to precede the clinical onset of peripartal diseases. Haptoglobin, an acute phase protein, has been proposed as a good indicator of inflammation in dairy cattle (Cray et al., 2009). Elevated serum haptoglobin concentrations in the first week postpartum have been reported for cows with fatty liver (Ametaj et al., 2005) and metritis (Huzzey et al., 2009; Dubuc et al., 2010). Similarly, we observed elevated haptoglobin concentrations in the first week postpartum in cows with ketosis, which is usually preceded by fatty liver (Veenhuizen et al., 1991), and metritis (Figures 4 and 5). In our study, sick cows in general had elevated haptoglobin concentrations in the first week postpartum (Figures 1 to 3). In support, cows that are clinically ill are known to have elevated haptoglobin concentrations (Hirvonen et al., 1997; Humblet et al., 2006). Therefore, haptoglobin concentrations during the first week postpartum may also indicate health status of dairy cows during early lactation.

Cows with subclinical or mild disease had less elevated serum haptoglobin concentrations in the first week postpartum than cows with severe diseases (Figures 1 to 5). Serum haptoglobin concentrations have been reported to reflect the disease severity for mastitis, metritis, and laminitis (Jawor et al., 2008; Huzzey et al. 2011; Pyörälä et al., 2011). Cows that were sold or died had elevated haptoglobin concentrations for a longer time period than cows

with severe disease. A prolonged inflammation period may be a risk indicator for mortality because inflammation causes extensive tissue damage and inhibits appetite. Therefore, haptoglobin concentrations in the first week postpartum may not only indicate current but also future disease severity.

Previous studies reported that the haptoglobin response differs depending on the bacterial source of infection (Hirvonen et al., 1999; Wenz et al., 2010; Pyröralä et al., 2011) and site of infection (Nielsen et al., 2004). The haptoglobin response, as indicated by peak concentrations and AUC values, may be proportional to the extent of tissue damage and the cytokine response to the infectious agent. In support, cows with more than one disease had or tended to have greater haptoglobin peak concentrations and AUC values in week 1 and 2 to 4 weeks postpartum than cows with only one disease (Figures 4 and 5). Furthermore, cows with metritis, ketosis, and mastitis had greater haptoglobin peak concentrations and AUC values in week 1 postpartum than cows with other diseases, and cows with mastitis had greater haptoglobin peak concentrations and AUC values in weeks 2 to 4 postpartum than cows with metritis (Figures 4 and 5). Differences in onset of diseases, infectious or inflammatory agent, or both may explain why the haptoglobin response differed among postpartal diseases.

Little research has been reported regarding the association between birth complications and serum haptoglobin concentrations. Cows with retained placenta, which is often associated with birth complications, are known to have elevated haptoglobin concentrations (Skinner et al., 1991; Crawford et al., 2005; Mordak et al., 2009). Similarly, cows with twins have been reported to have elevated haptoglobin concentrations postpartum (Crawford et al., 2005). In our study, cows with birth complications did not only have greater haptoglobin concentrations in the

first week postpartum, but also in the last weeks prepartum (Figures 6 and 7). Therefore, elevated haptoglobin concentrations may precede birth complications.

Similarly, little research has been reported whether an increase in serum haptoglobin concentrations precede the onset of diseases. Huzzey et al. (2009) and Dubuc et al. (2010) reported that elevated serum haptoglobin concentrations precede clinical signs of metritis. In a follow-up study, however, Huzzey et al. (2011) reported that haptoglobin concentrations in the last three weeks prepartum are not associated with incidence and severity of disease in the first four weeks postpartum. Grouping cows according to the first treatment time allowed us to look at specific time periods before clinical diagnosis and treatment of disease. Haptoglobin concentrations were elevated during the last week before diagnosis and treatment of disease (Figures 8 to 10). Thus, elevated haptoglobin concentrations may precede clinical onset of disease, and therefore may assist in early detection and treatment of sick cows.

A strength of the current study was that multiple blood samples were collected and analyzed throughout the periparturient period, which allowed us to calculate AUC values and decreased the measurement error. Another strength of the study was that the health of the cows was checked twice daily and included multiple cervical palpations, serum BHBA, and milk somatic cell concentrations. There are several potential limitations to our study. Diseases were generally diagnosed and treated by the farm manager and not a veterinarian. The study did not include primiparous cows, which may limit our findings to multiparous cows. Primiparous cows may have a greater haptoglobin response after calving than multiparous cows (Crawford et al., 2005; Saremi et al., 2012). We restricted our study to multiparous cows because of the greater incidence of diseases. The study was limited to one commercial farm that milked

frequently. A lower number of milkings could have decreased stress and serum haptoglobin concentrations.

In conclusion, elevated serum haptoglobin concentrations were associated with disease status, severity, number, and type and preceded birth complications and clinical onset of disease in early lactation. Thus, serum haptoglobin concentrations may assist in early detection and treatment of diseases in early lactation. Future studies at a larger scale and at various farms that differ in management protocols are required to confirm our findings before definite conclusions can be drawn.

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References

- Ametaj, B.N., B.J. Bradford, G. Bobe, R.A. Nafikov, Y. Lu, J.W. Young, and D.C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci.* 85: 165-175.
- Ametaj, B.N., A. Hosseini, J.F. Odhiambo, S. Iqbal, S. Sharma, Q. Deng, T.H. Lam, U. Farooq, Q. Zebeli, and S.M. Dunn. 2011. Application of acute phase proteins for monitoring inflammatory states in cattle, acute phase proteins as early non-specific biomarkers of human and veterinary diseases. Prof. Francisco Veas (Ed.), ISBN: 978-953-307-873-1, InTech. <http://www.intechopen.com/books/acute-phase-proteins-for-monitoring-inflammatory-states-in-cattle>.
- Crawford, R.G., K.E. Leslie, R. Bagg, C. P. Dick, and T.F. Duffield. 2005. The impact of controlled release capsules of monensin on postcalving haptoglobin concentrations in dairy cattle. *Can. J. Vet. Res.* 69: 208-214.
- Cray, C., J. Zaias, and N.H. Altman. 2009. Acute phase response in animals: a review. *J. Am. Assoc. Lab. Anim. Sci.* 59: 517-526.
- Dubuc, J., T.F. Duffield, K.E. Leslie, J.S. Walton, and S.J. LeBlanc. 2010. Risk factors for postpartum uterine diseases in dairy cows. *J. Dairy Sci.* 93: 5764-5771.

- Hirvonen, J., K. Eklund, A.M. Teppo, G. Huszenicza, M. Kulcsar, H. Saloniemi, and S. Pyörälä. 1999. Acute phase response in dairy cows with experimentally induced *Escherichia coli* mastitis. *Acta Vet. Scand.* 40: 35-46.
- Horadagoda, N.U., K.M.G. Knox, H.A. Gibbs, S.W.J. Reid, A. Horadagoda, S.E.R. Edwards, and P.D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet. Rec.* 144: 437-441.
- Humblet, M.F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J.M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. *Vet. Clin. Path.* 35: 188-193.
- Huzzey, J.M., T.F. Duffield, S.J. LeBlanc, D.M. Veira, D.M. Weary, and M.A.G. von Keyserlingk. 2009. Short communication: haptoglobin as an early indicator of metritis. *J. Dairy Sci.* 92: 621-625.
- Huzzey, J.M., D.V. Nydam, R.J. Grant, and T.R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. *J. Dairy Sci.* 94: 5878-5889.
- Jacobsen, S., P.H. Andersen, T. Toelboell, and P.M.H. Heegaard. 2004. Dose dependency and individual variability of the lipopolysaccharide induced bovine acute phase protein response. *J. Dairy Sci.* 87: 3330-3339.
- Jawor, P., S. Steiner, T. Stefaniak, W. Baumgartner, and A. Rzasa. 2008. Determination of selected acute phase proteins during the treatment of limb diseases in dairy cows. *Veterinari Medicina* 53: 173-183.

- LeBlanc, S. 2010. Health in the transition period and reproductive performance. *Adv. Dairy. Tech.* 2297-110.
- Mordak, R. 2009. Postpartum serum concentration of haptoglobin in cows with fetal membranes retention. *Cattle. Pract* 17: 100-102.
- National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Acad. Sci., Washington, DC.
- Nielsen, B.H., S. Jacobsen, P.H. Andersen, T.A. Niewold, and P.M.H. Heegaard. 2004. Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions. *Vet. Rec.* 154: 361-365.
- Petersen, H. H., J.P. Nielsen, and P.M.H. Heegaard. 2004. Application of acute phase protein measurement in veterinary clinical chemistry. *Vet. Res.* 35:163–187.
- Pyörälä. S., M. Hovinen, H. Simojoki, J. Fitzpatrick, P.D. Eckersall, and T. Orro. 2011. Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. *Vet. Rec.* 168: 535.
- SAS Institute. 2001. SAS User's Guide. Statistics, Version 9.1. SAS Inst. Inc., Cary, NC.
- Saremi, B., A. Al-Dawood, S. Winand, U. Müller, J. Pappritz, D. von Soosten, J. Rehage, S. Dänicke, S. Häussler, M., Mielenz, and H. Sauerwein. 2012. Bovine haptoglobin as an adipokine: Serum concentrations and tissue expression in dairy cows receiving a conjugated linoleic acids supplement throughout lactation. *Vet. Immunol. Immunopathol.* 146: 201-211.

Skinner, J.G., R.A.L. Brown, and L. Roberts. 1991. Bovine haptoglobin response in clinically defined field conditions. *Vet. Rec.* 128: 147-149.

Veenhuizen, J.J., J.K. Drackley, M.J. Richard, T.P. Sanderson, L.D. Miller, and J.W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. *J. Dairy Sci.* 74: 4238-4253.

Vels, L., C.M. Rontved, M. Bjerring, and K.L. Ingvarsten. 2009. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. *J. Dairy Sci.* 92: 922-934.

Wenz, J.R., L.K. Fox, F.J. Muller, M. Rinaldi, R. Zeng, and D.D. Bannerman. 2010. Factors associated with concentrations of select cytokine and acute phase proteins in dairy cows with naturally occurring clinical mastitis. *J. Dairy Sci.* 93: 2458-2470.

Figures

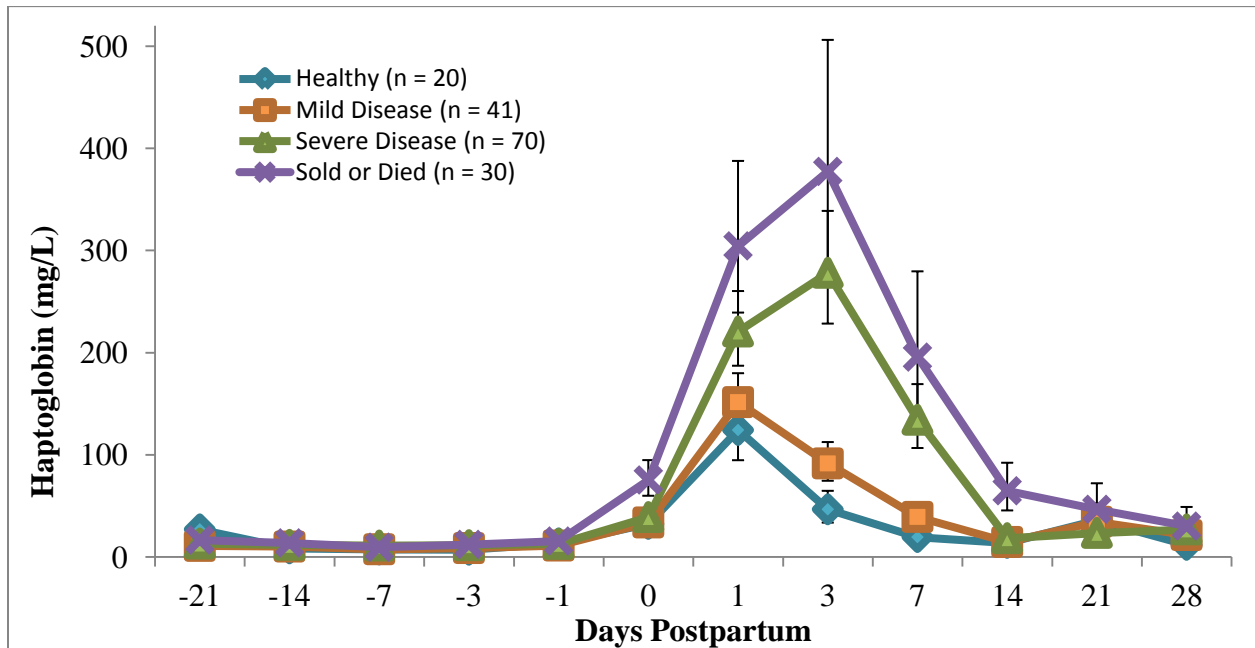


Figure 1: Elevated serum haptoglobin concentrations during the first week postpartum indicate disease status and severity of dairy cows during the peripartal period.

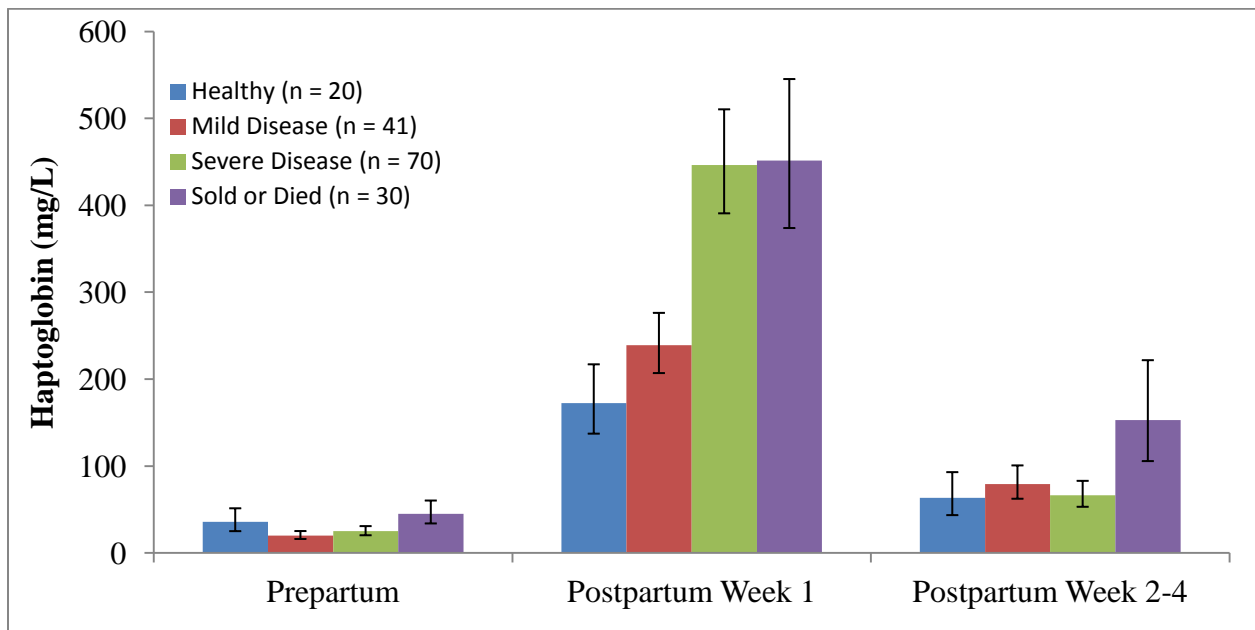


Figure 2: Compared to healthy cows, cows with clinical or subclinical diseases had greater peak concentrations in the first week after calving.

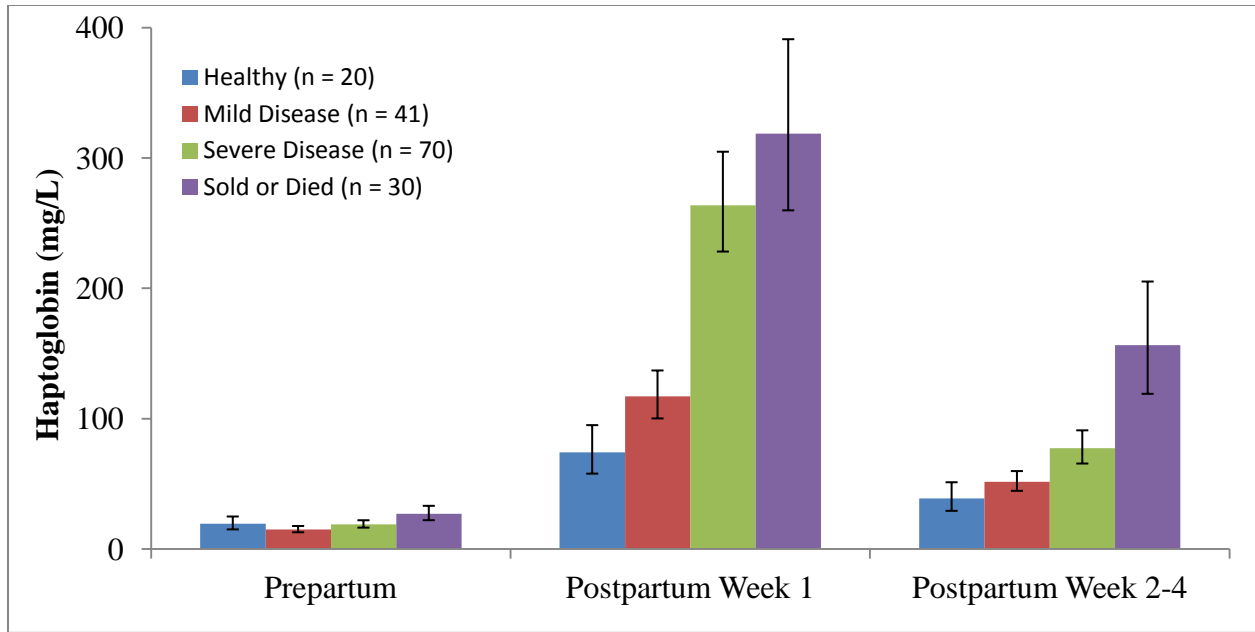


Figure 3: Compared to healthy cows, cows with clinical or subclinical diseases had greater AUC in the first week after calving.

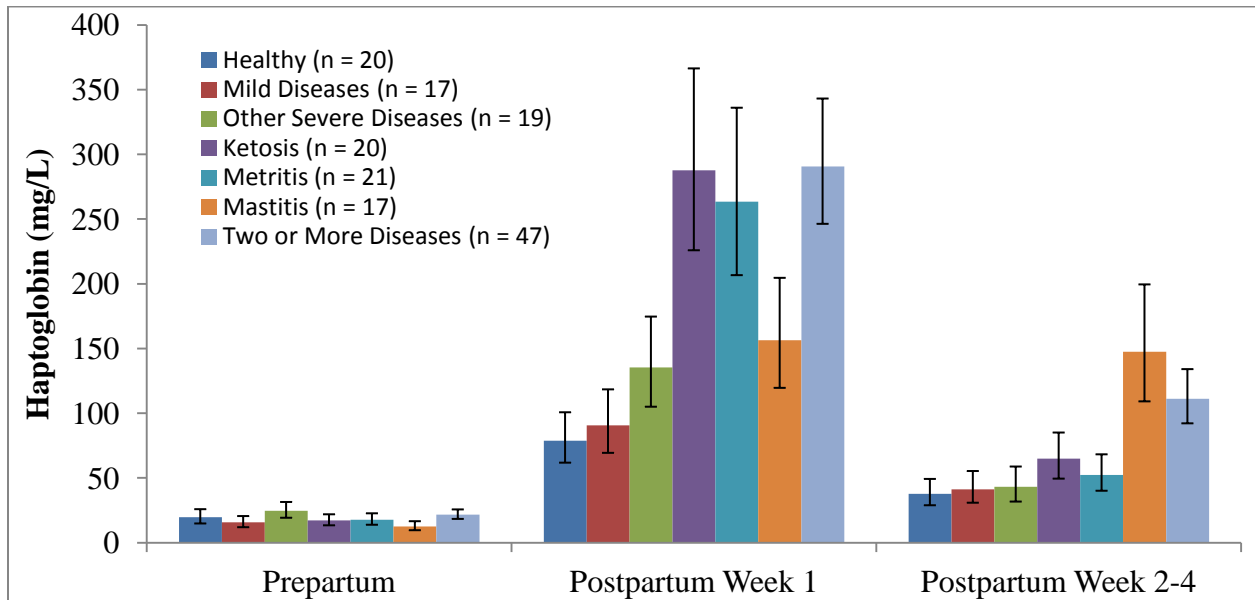


Figure 4: The association between serum haptoglobin concentrations and peripartur diseases differed between number and type of diseases. Total haptoglobin concentrations during week 1 and week 2 to 4 after calving were indicators for number and type of diseases.

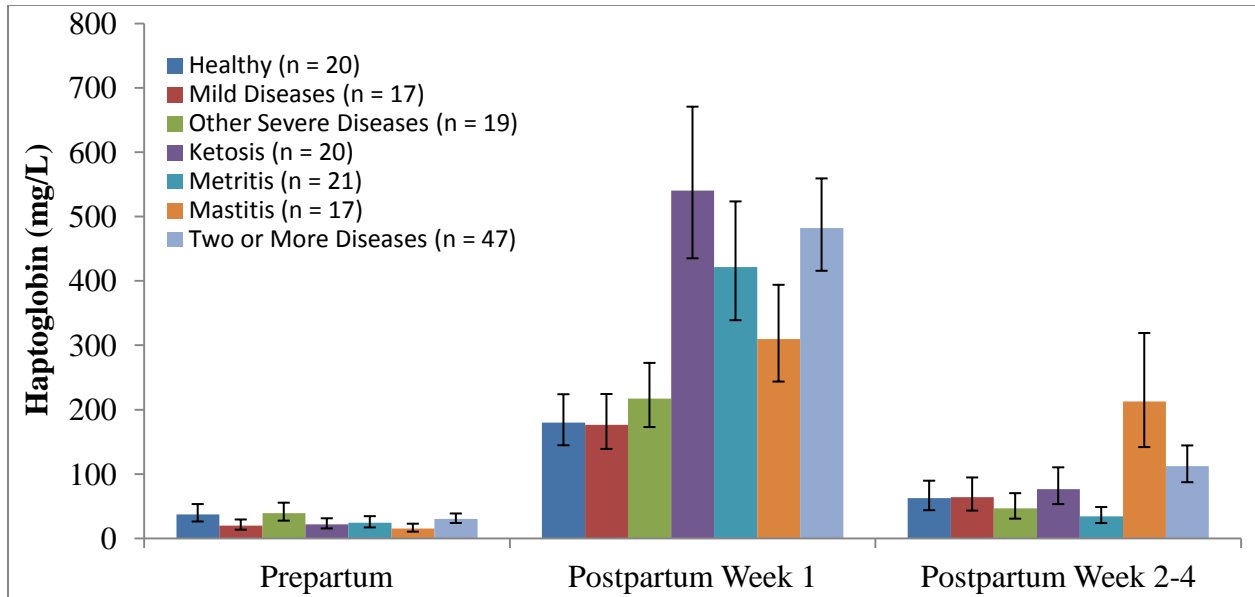


Figure 5: The association between serum haptoglobin concentrations and peripartur diseases differed between number and type of diseases. Total peak haptoglobin concentrations during week 1 and week 2 to 4 after calving were indicators for number and type of diseases.

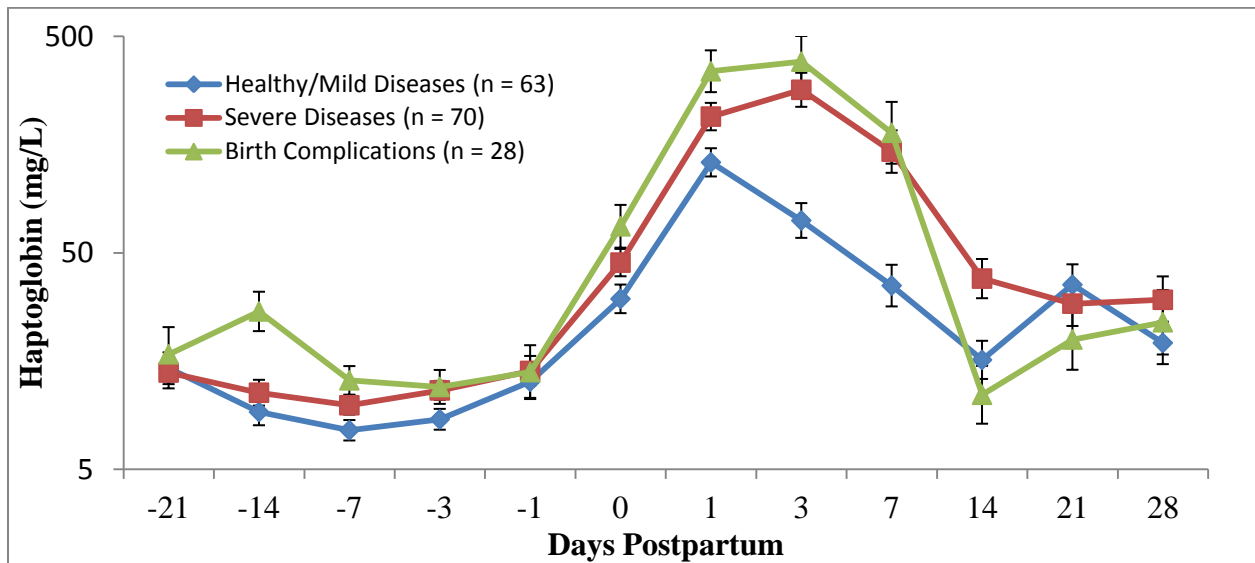


Figure 6: Elevated serum haptoglobin concentrations were associated with birth complications (twins, hard pull, or both). Cows with versus without birth complications did not only have greater haptoglobin concentrations in the first week postpartum but also prepartum at day -14.

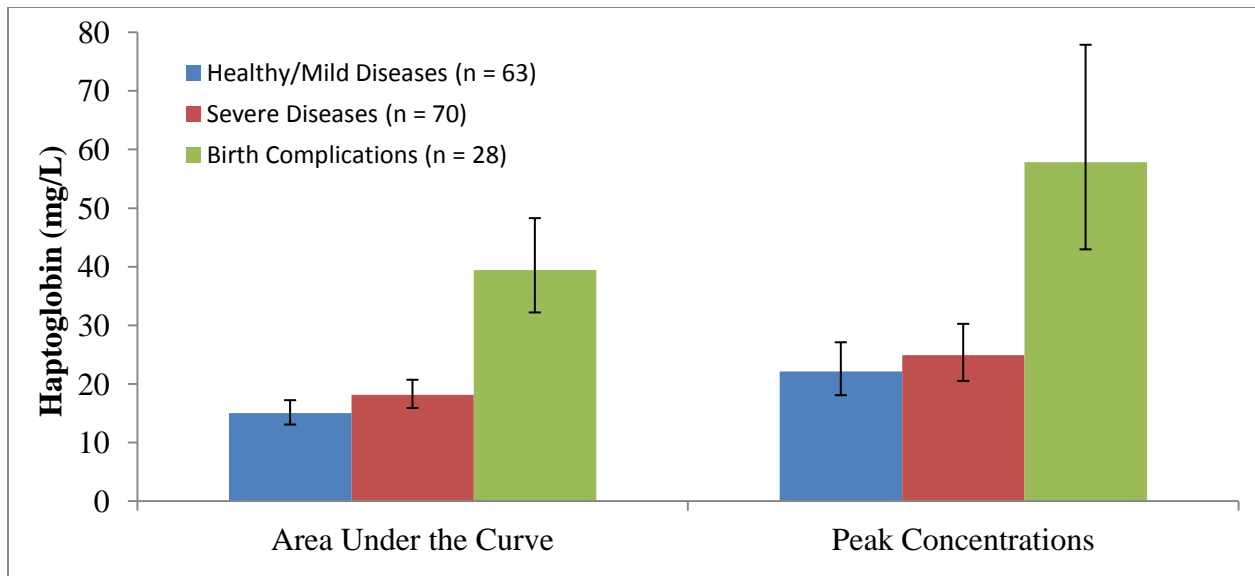


Figure 7: Elevated serum haptoglobin concentrations were associated with birth complications (twins, hard pull, or both). Compared to cows without birth complications, cows with birth complications had greater peak concentrations and AUC values in the last 3 weeks prepartum.

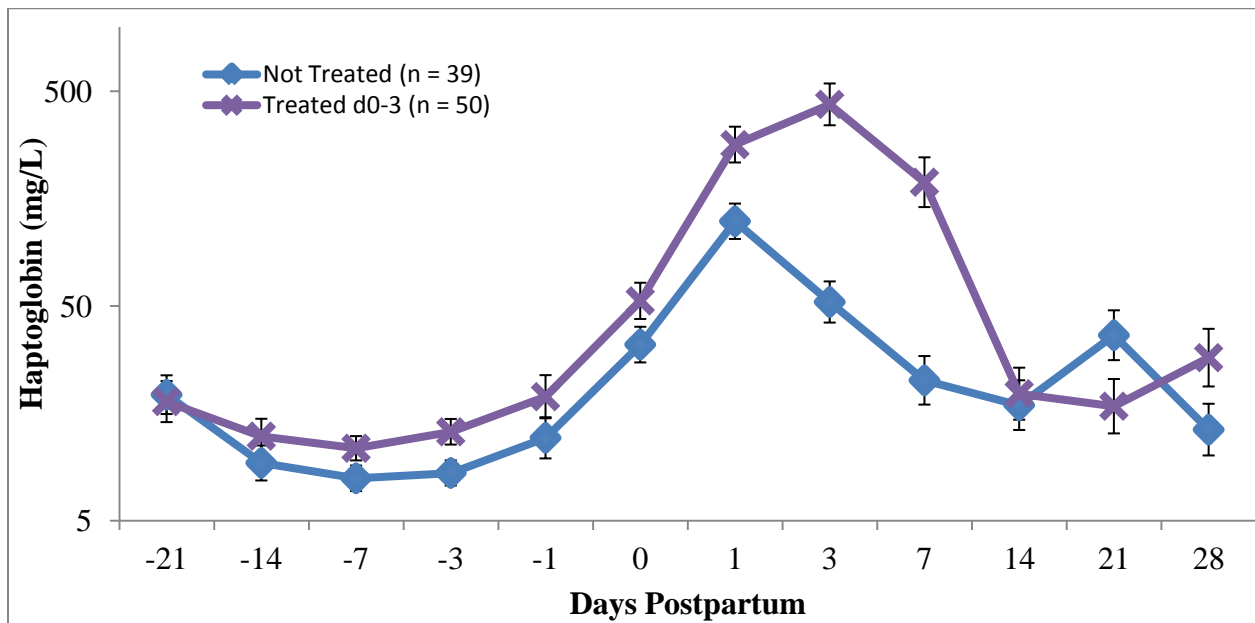


Figure 8: Cows that were treated first within day 0 to 3 postpartum had greater haptoglobin concentrations at day -7 and -3 postpartum.

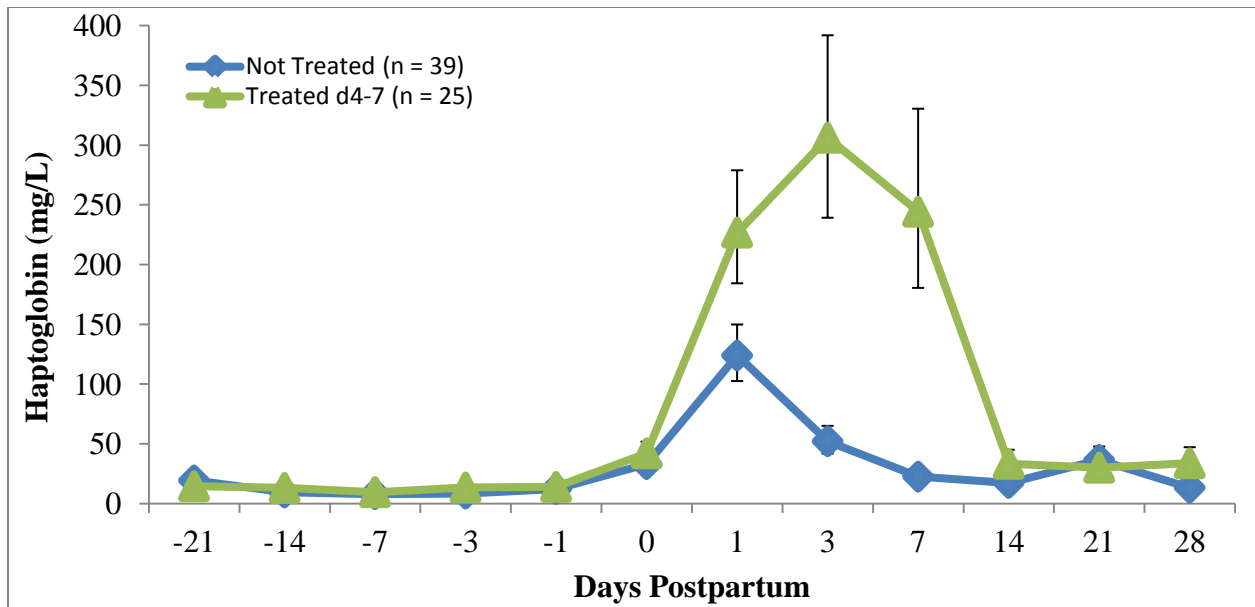


Figure 9: Cows that were treated first between 4 and 7 days postpartum had greater haptoglobin concentrations at day 3 postpartum.

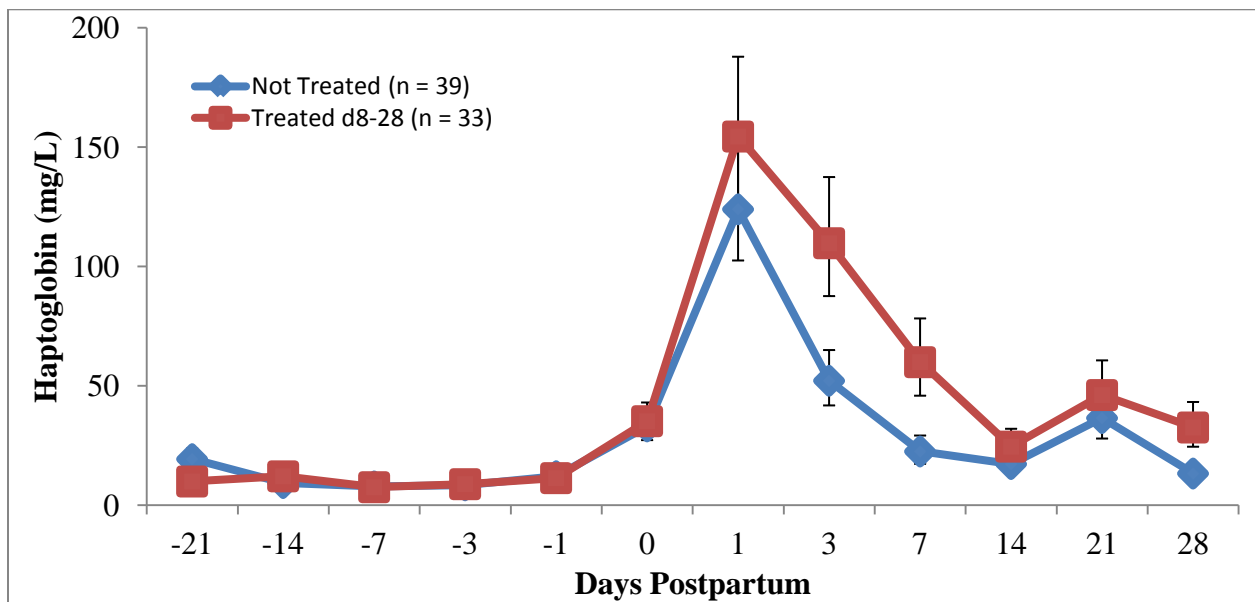


Figure 10: Cows that were treated first between 8 and 28 days postpartum had greater haptoglobin concentrations at day 3 and 7 postpartum.