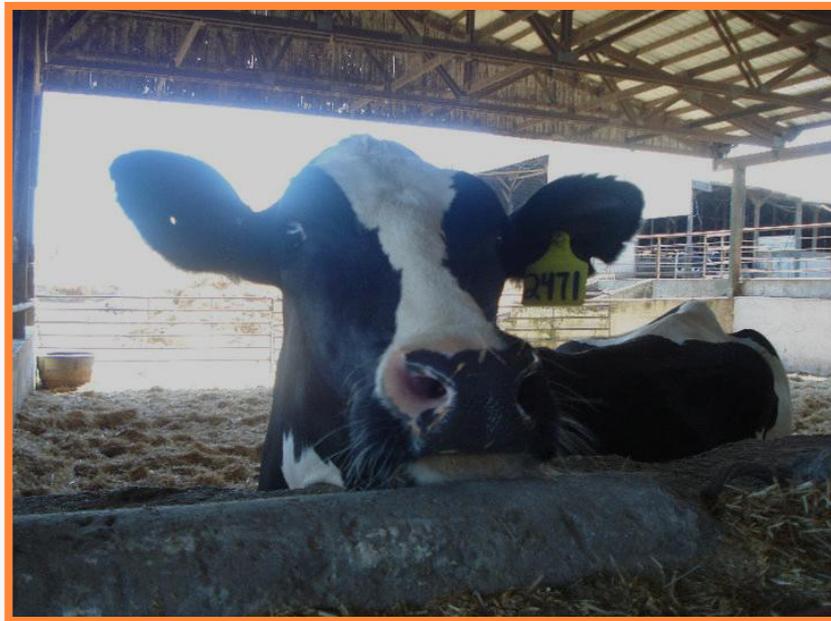


Serum Haptoglobin as an Indicator for Calving Difficulties and Postpartal Diseases in Transition Dairy Cows



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TRANSITION PERIOD

- 3 weeks from expected calving date to 3 weeks postpartum
 - Elevated incidence of metabolic and infectious diseases
 - Increased exposure and susceptibility of the mammary gland and uterine tract to bacteria



Transition Period

20 % older cows die

90% become ill

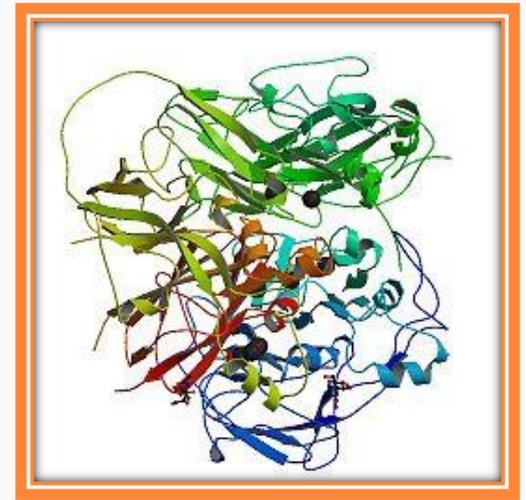
Milk Loss and Treatment Costs

Cow Quality of Life decreased
Profit and Milk Quality decreased



HAPTOGLOBIN

- Acute Phase Protein
- Primary synthesis in the liver
 - Secondary synthesis in various body tissues
 - Mammary gland
 - White blood cells
 - Adipose tissue
 - Ovaries



ACUTE PHASE RESPONSE

- Body's response to infectious agents that can cause stress, trauma, and inflammation
 - Innate immune system
- Haptoglobin primarily serves to prevent further tissue damage and promote repair
 - Proportional to severity of challenge



Antioxidant

Immunomodulator

**Anti-
Inflammatory
Agent**

Functions of Haptoglobin

**Prevents future tissue damage and promotes
tissue generation**

Bacteriostat

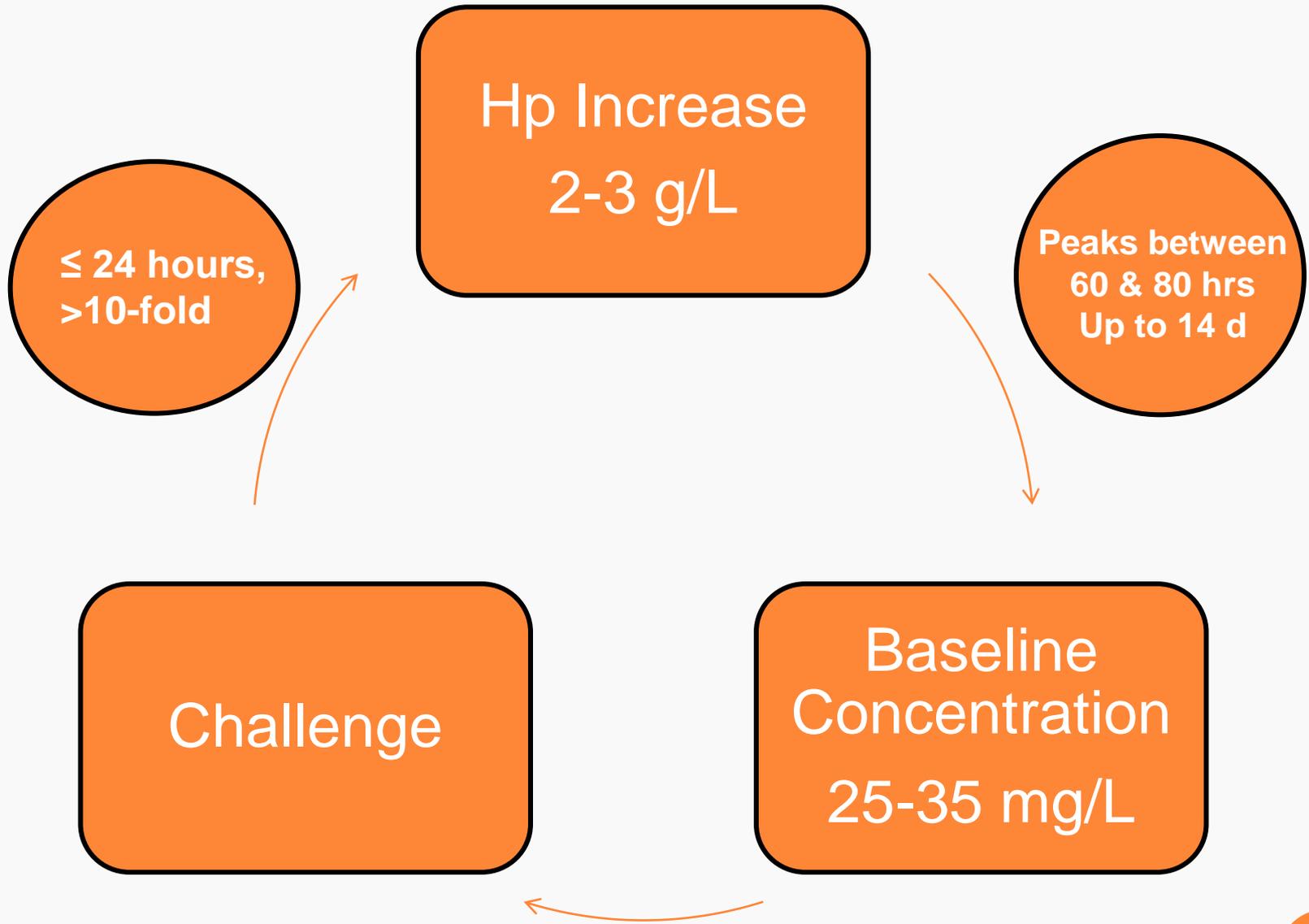
**Tissue-
Regeneration
Agent**



HAPTOGLOBIN IN BOVINE

- Proposed as an indicator of acute and chronic diseases
- Limited sensitivity (percent of animals detected as sick)
 - Delayed reaction (24 hr) to tissue damage or infection
 - Decreases after an acute infection
 - Does not always go up during disease





Objective 1

Evaluate whether peripartal [Hp] were associated with:

- Health status and severity
- Type and number of diseases

Objective 2

- Examine whether prepartal [Hp] indicate birth complications
- Examine whether [Hp] were elevated prior to clinical signs of diseases



Hypothesis 1

Haptoglobin concentrations will increase in the peripartal period:

- In relation to health status, severity, type and number of diseases

Hypothesis 2

Haptoglobin concentrations will increase in the peripartal period:

- Prepartum in cows that had birth complications
- Prior to the onset of clinical signs of diseases



METHODS



- Van Beek Dairy in Monroe, Oregon, in Spring and Summer of 2010
- 161 multiparous Holstein cows
- 4 weeks prior to expected calving date to 4 weeks post-calving



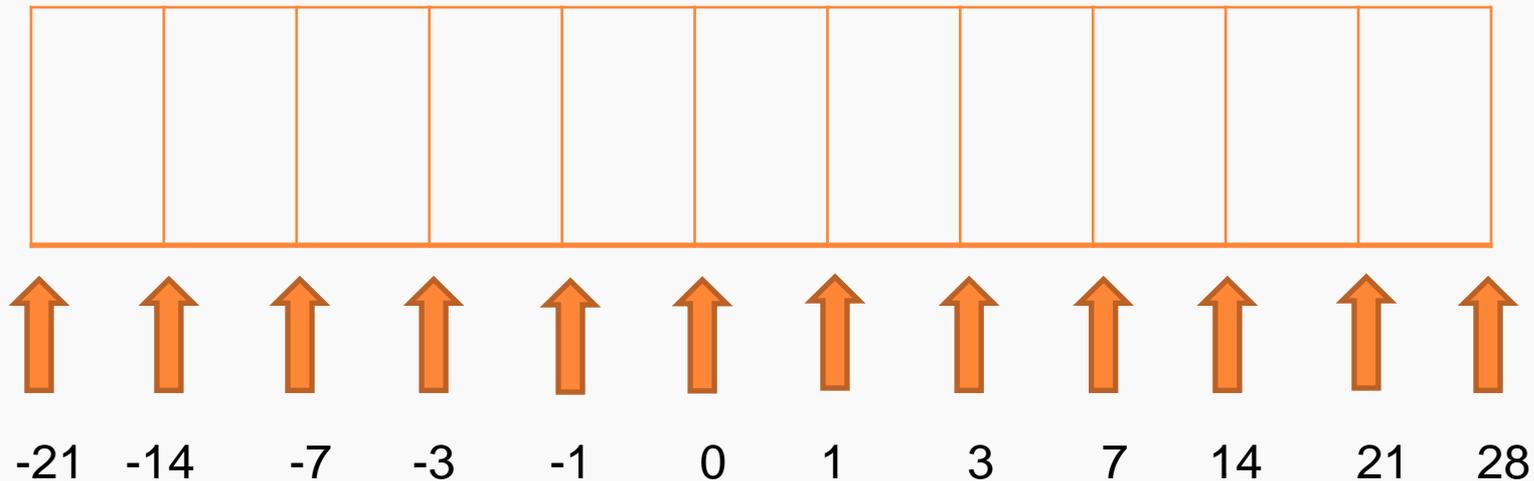
METHODS: ANIMAL MANAGEMENT

- Between days -28 and 100 postpartum, cows were monitored daily for signs of diseases
- Medical treatment was provided and recorded by herd manager and recorded in Dairy Comp (Valley Ag. Software, Inc., Tulare, CA)



BLOOD COLLECTION

- Blood samples were taken according to the figure below (0 = day of calving)



BLOOD COLLECTION

- 5-8 mL of blood was taken from the coccygeal vein or artery in a 10 mL serum vacutainer tube
- Samples were placed on ice and transported to lab
 - Serum was separated by centrifugation at room temperature for 20 minutes at 1600 x g
 - Stored at -20 C until chemical analysis







BLOOD ANALYSIS

- Samples were analyzed using a bovine haptoglobin enzyme-linked immunosorbent assay (ELISA)
 - Life Diagnostics, Inc., Catalog number: 2410-7
- Procedure was conducted according to manufacturer's instructions



CLASSIFICATION OF GROUPS

○ Disease Status and Severity

Healthy (n=19)

- No medical treatment
- SCC < 1,000,000 cells/mL
and
- BHBA < 1.3 mmol/L

Mild Disease (n=49)

- Treated but no glucose precursors or antibiotics,
- SCC > 1,000,000 cells/mL
or
- BHBA > 1.3 mmol/L

Severe Disease (n=63)

- Treated with antibiotics with withdrawal period
- Oral or I.V. glucose precursors

Died/Sold (n=30)

- Died or sold in the first 100 days postpartum



Healthy (n=20)

No medical treatment

Mild Disease
(n=17)

Treated without glucose precursors

Other Diseases
(n=19)

Diseases other than ketosis, metritis,
or mastitis

Ketosis(n=20)

BHBA > 1.3 mMol/L

Metritis(n=21)

Placental retention or purulent/putrid
vaginal or cervical discharge

Mastitis (n=17)

Milk flakes, swelling, or SCC >
1,000,000 cells/mL

2+ Diseases
(n=47)

Cows with more than 1 disease

CLASSIFICATION OF GROUPS

○ Birth Complications

Healthy (n=63)

- Healthy cows or cows with mild diseases

Other Severe Disease (n=70)

- Severe disease without birth complications

Birth Complications (n=28)

- Twinning (n=16)
- Hard pull or C-Section (n=8)
- Both (n=4)

CLASSIFICATION OF GROUPS

- First Treatment Time

No Treatment (n=39)

Treated
D-21 to -1
(n=14)

Treated
D0 to 3
(n=50)

Treated
D4 to 7
(n=25)

Treated
D8 to 28
(n=28)

SUMMARY OF CLASSIFICATION GROUPS

- Disease Status and Severity
- Disease Number and Type
- Birth Complications
- First Treatment Time



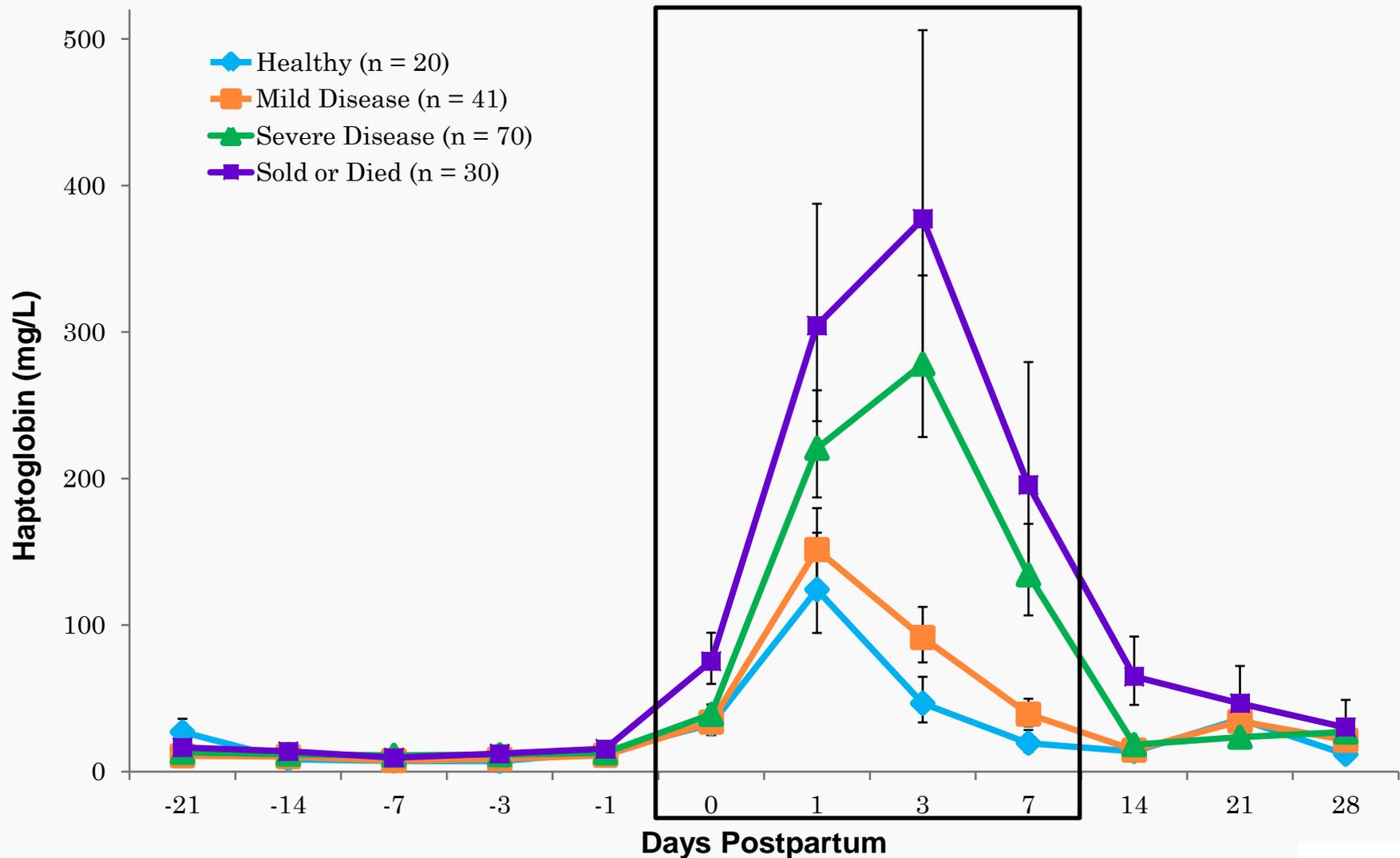


Figure 1: Elevated serum haptoglobin concentrations during the first week postpartum indicate disease status and severity of dairy cows during the peripartal period. Cows in the two severe groups had greater [Hp] than mild diseases ($P < 0.001$).

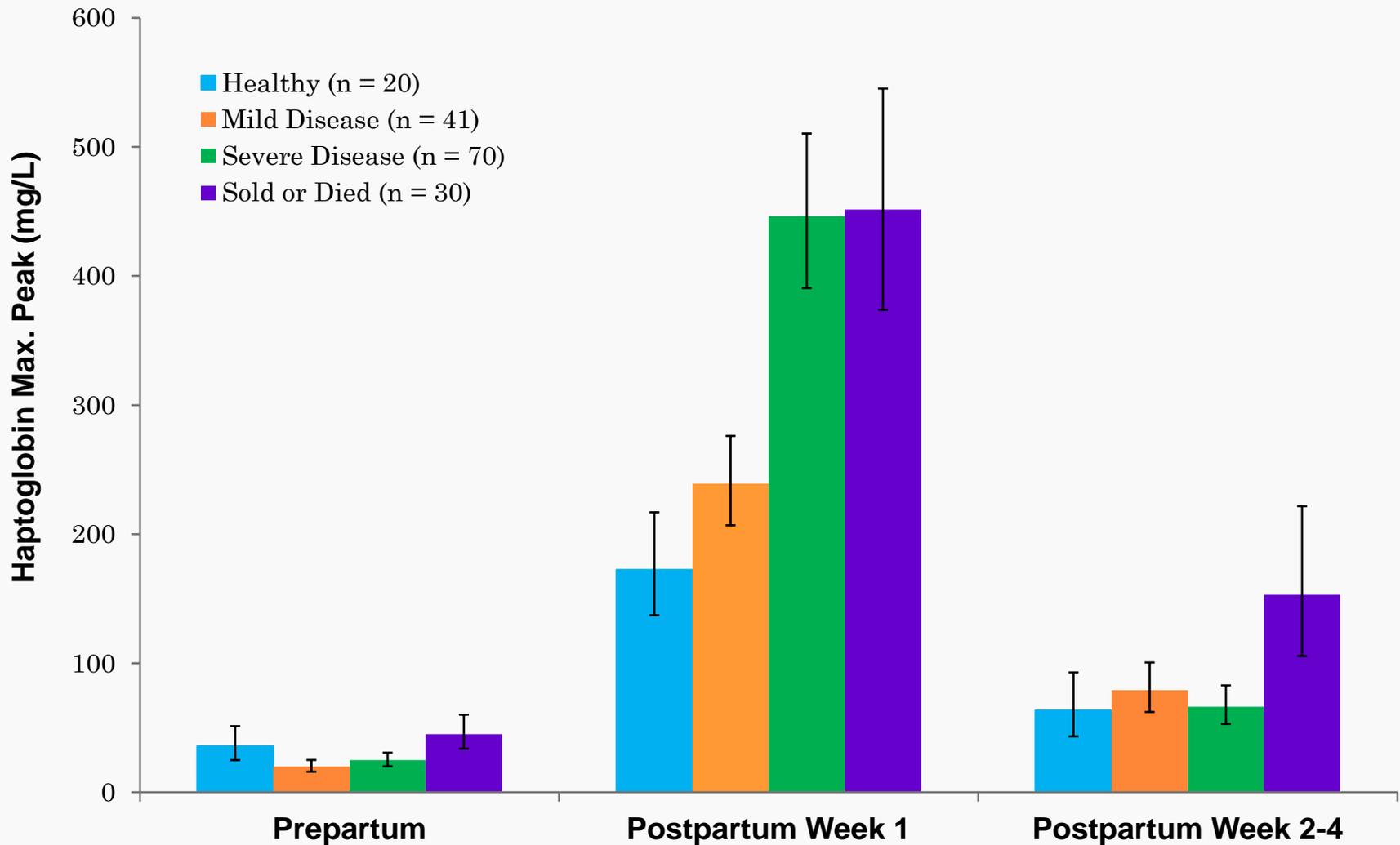


Figure 2: Compared to healthy cows, sick cows had greater peak [Hp] in the first wk after calving ($P < 0.001$). Cows with severe diseases had greater peak [Hp] than the mild/healthy groups in the first wk after calving ($P < 0.001$). Cows that were sold or died had greater peak [Hp] than cows with severe disease in wk 2 to 4 postpartum ($P = 0.04$).

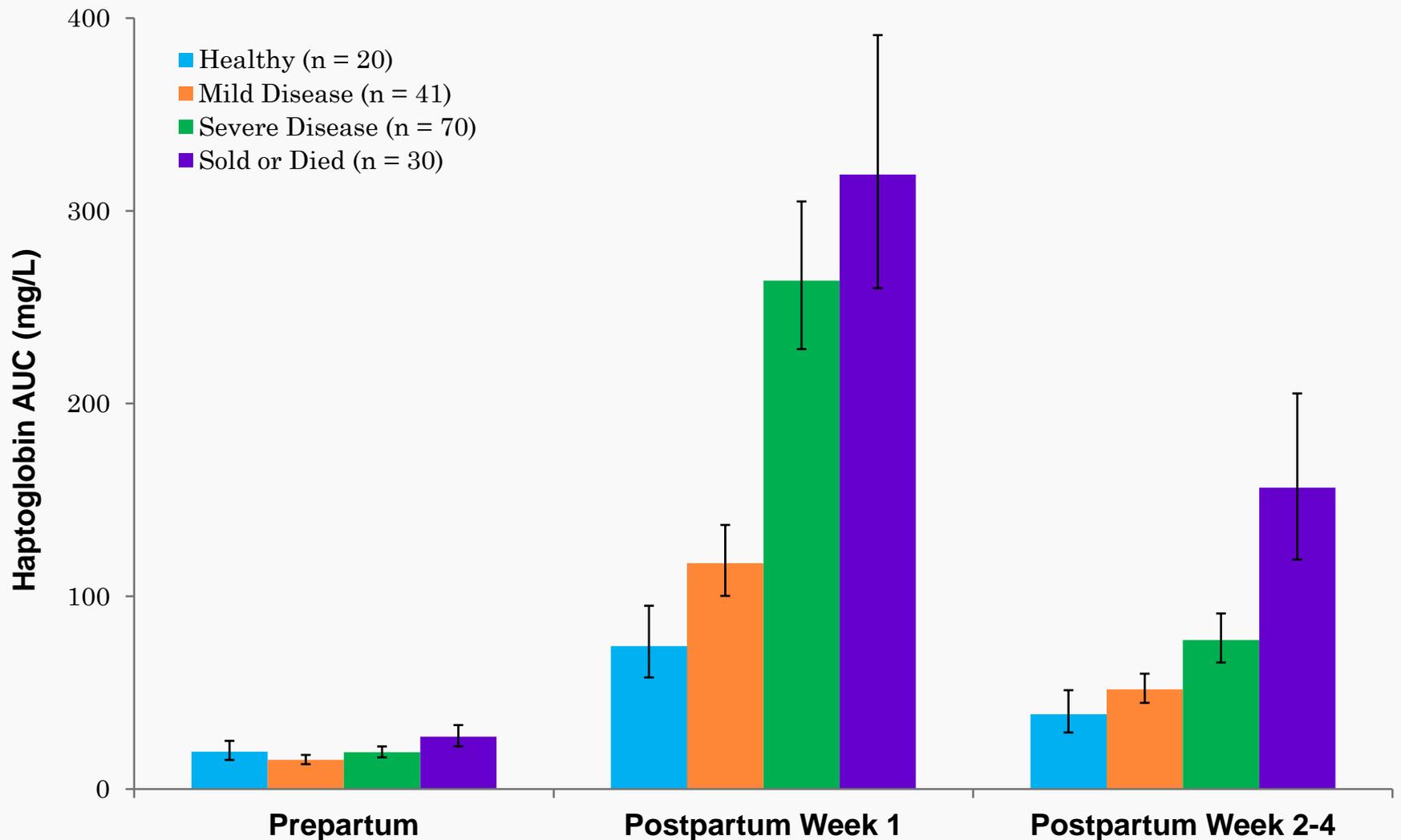


Figure 3: Compared to healthy cows, sick cows had greater [Hp] AUC values in the first wk postpartum ($P < 0.001$). Cows with severe diseases had greater [Hp] AUC values than the mild/healthy groups in the first wk postpartum ($P < 0.001$). Cows that were sold or died had greater [Hp] AUC values than cows with severe disease in wk 2 to 4 postpartum ($P = 0.02$).

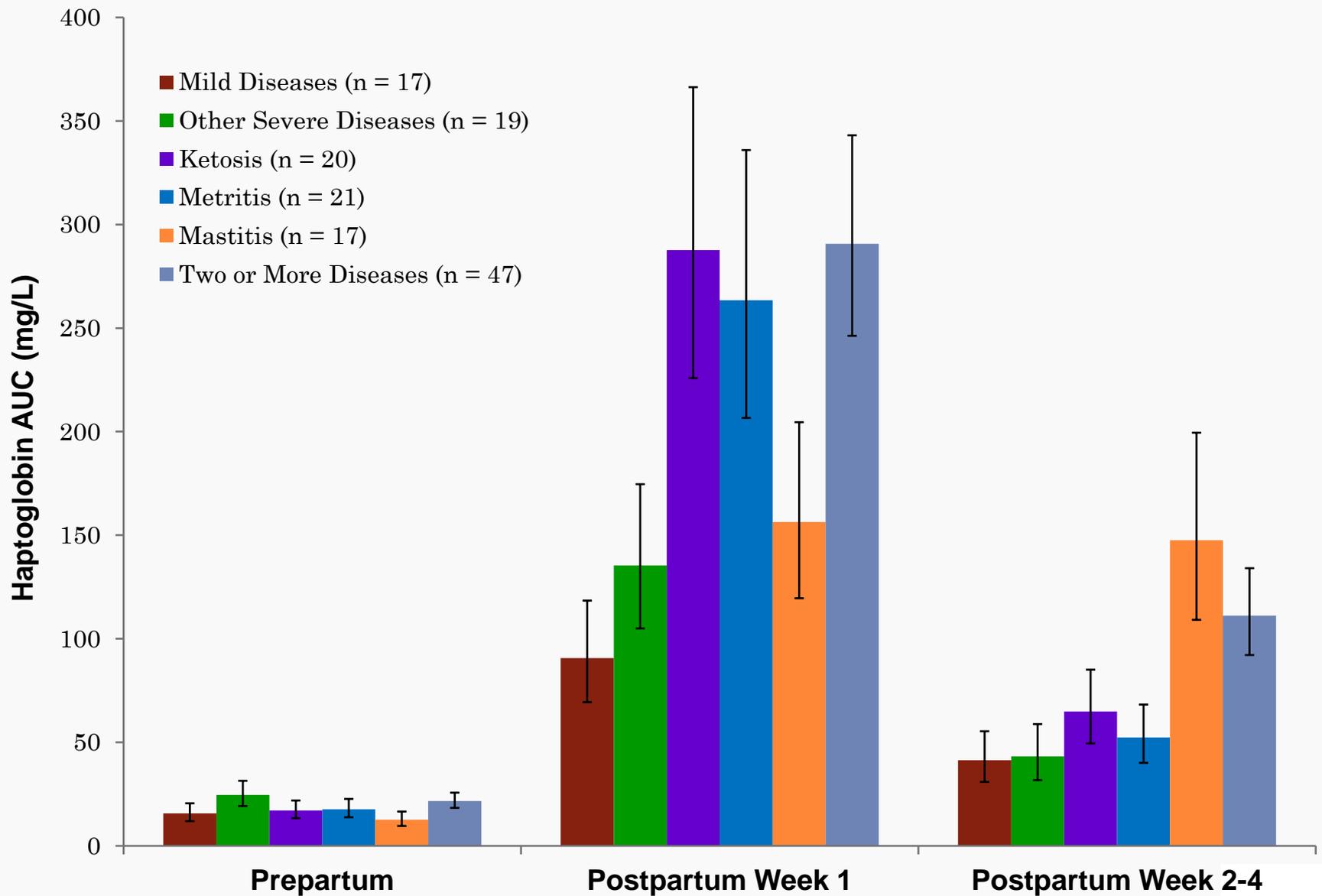


Figure 4: Disease number and type affect [Hp] AUC values. Cows with ketosis, metritis, and 2 or more diseases had the greatest [Hp] AUC values in wk 1 postpartum. Cows with mastitis and 2 or more disease had the greatest [Hp] AUC values in wk 2 to 4 postpartum.

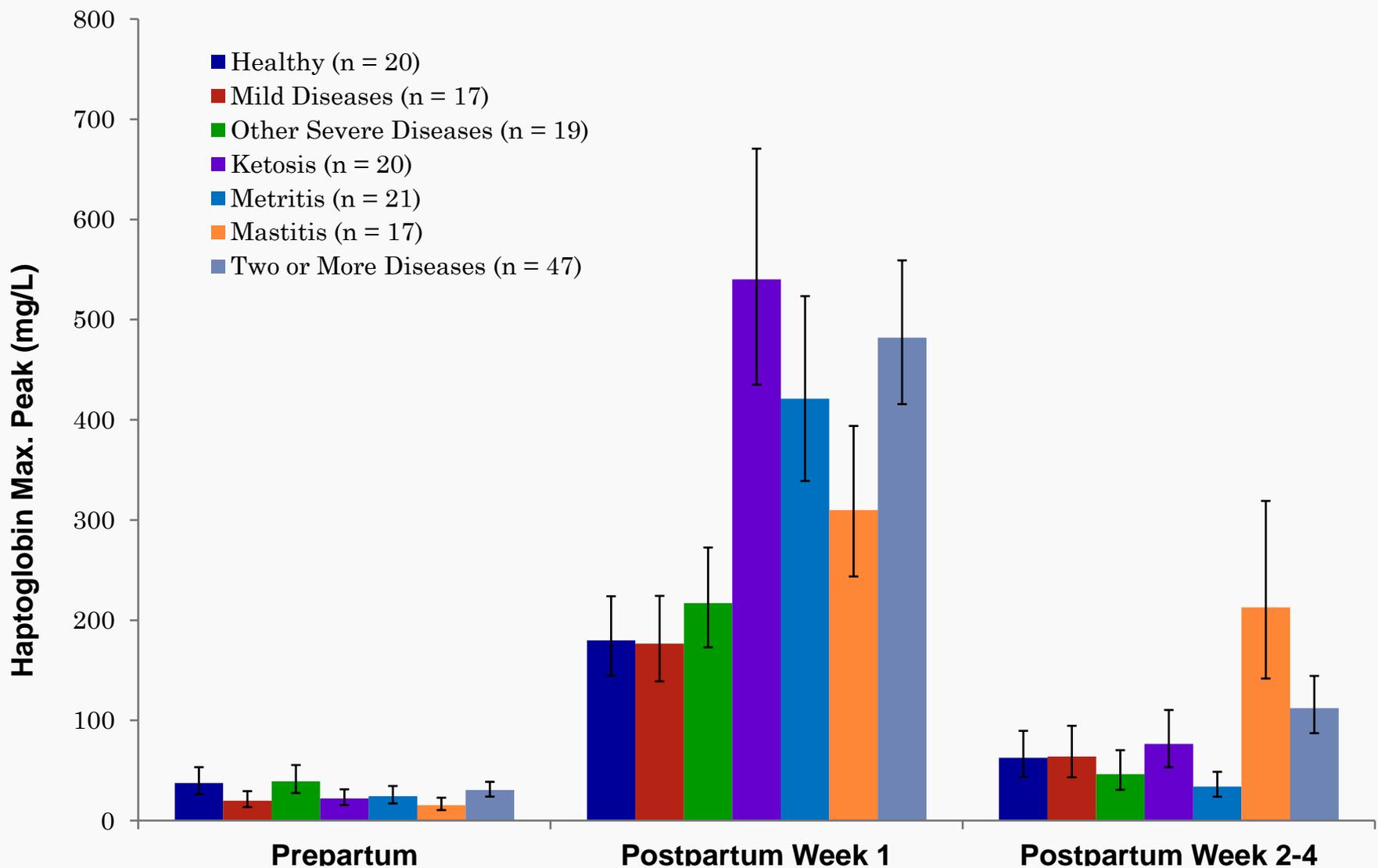


Figure 5: Disease number and type affect peak [Hp]. Cows with ketosis, metritis, and 2 or more diseases had the greatest peak [Hp] in wk 1 postpartum. Cows with mastitis and 2 or more disease had the greatest peak [Hp] in wk 2 to 4 postpartum. .

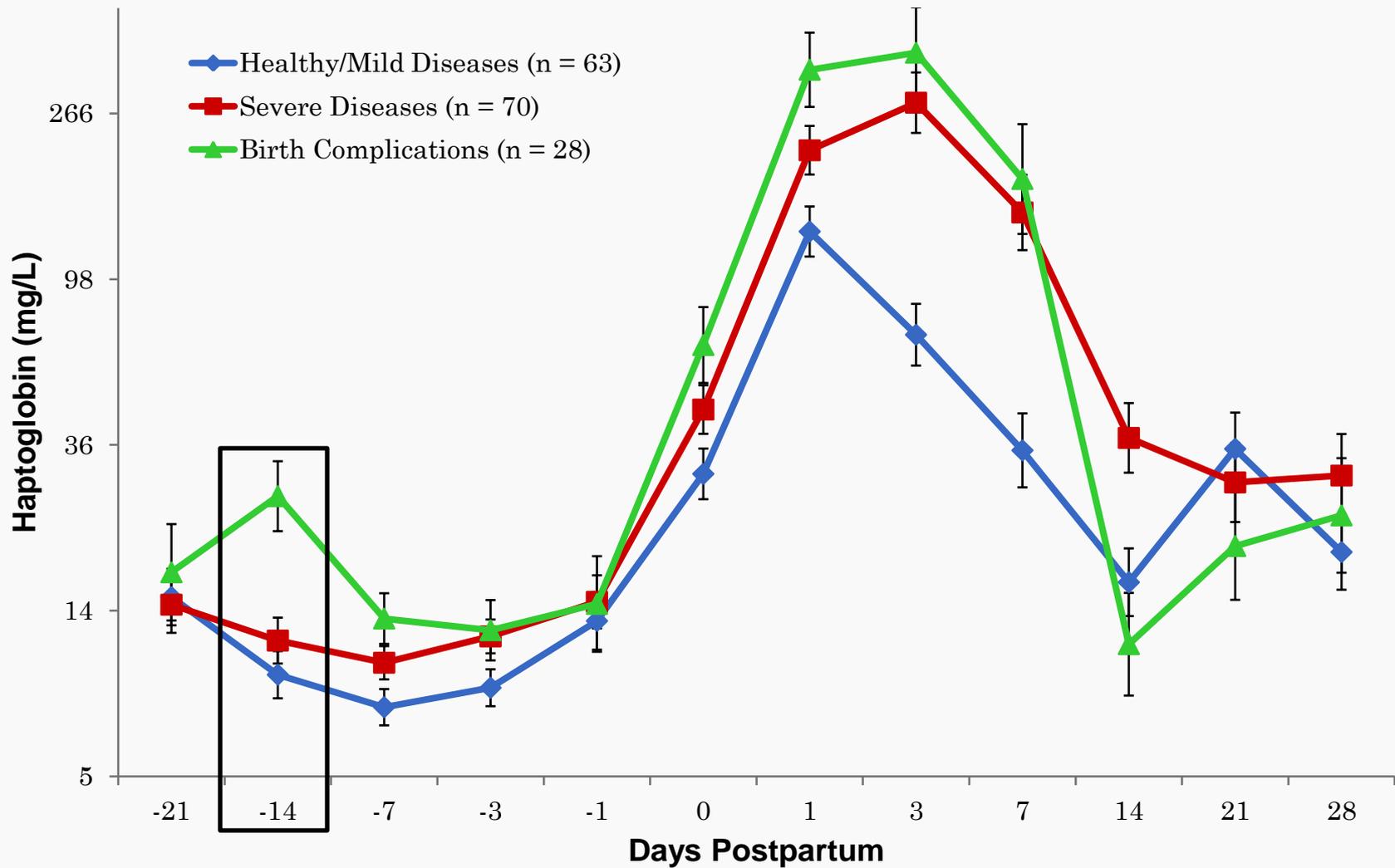


Figure 6: Cows with versus without birth complications had greater [Hp] at days -14 prepartum ($P < 0.001$)

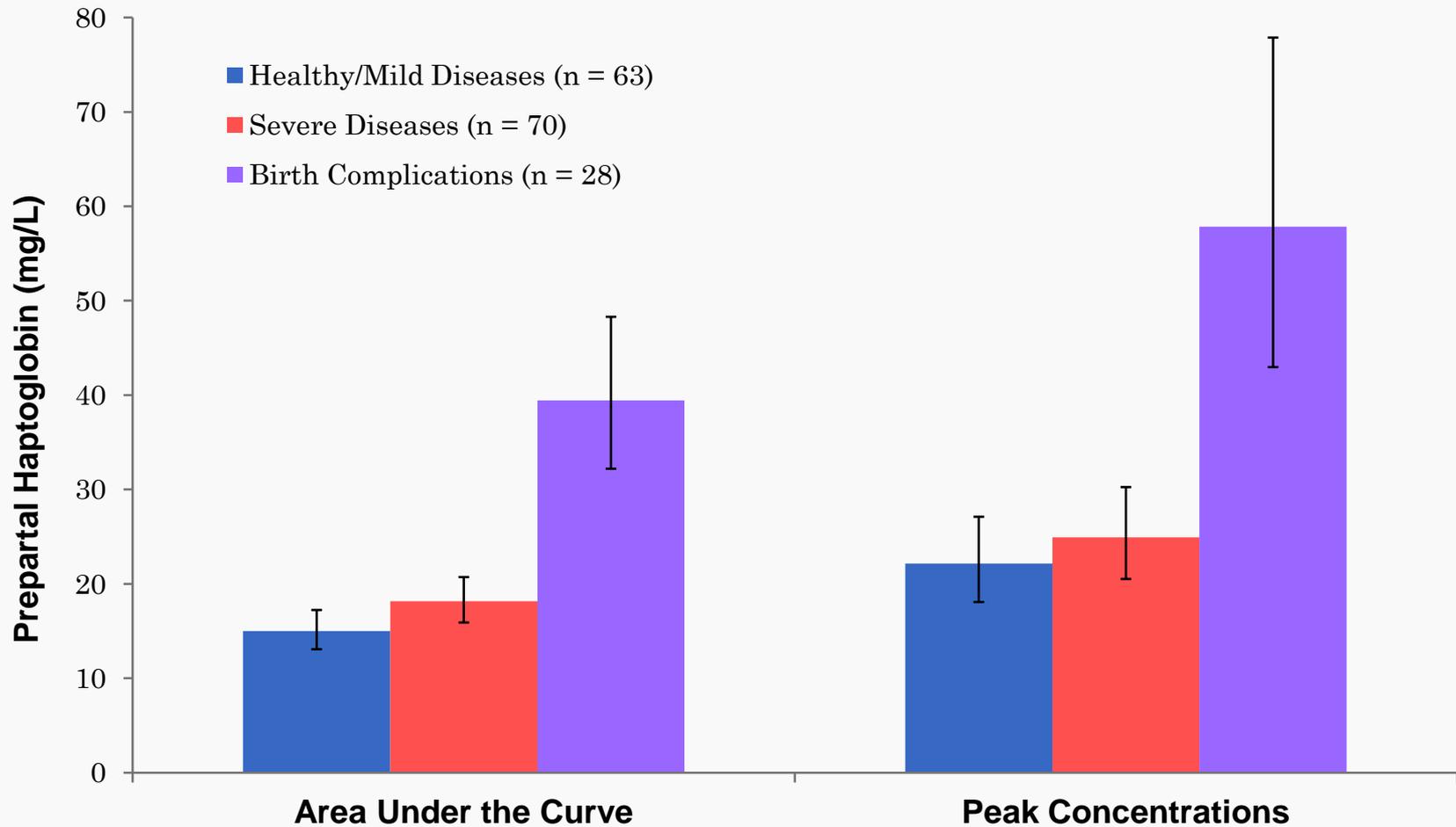


Figure 7: Compared to cows without birth complications, cows with birth complications had greater [Hp] AUC values ($P < 0.001$) and peak concentrations ($P = 0.004$) in the last 3 wks prepartum

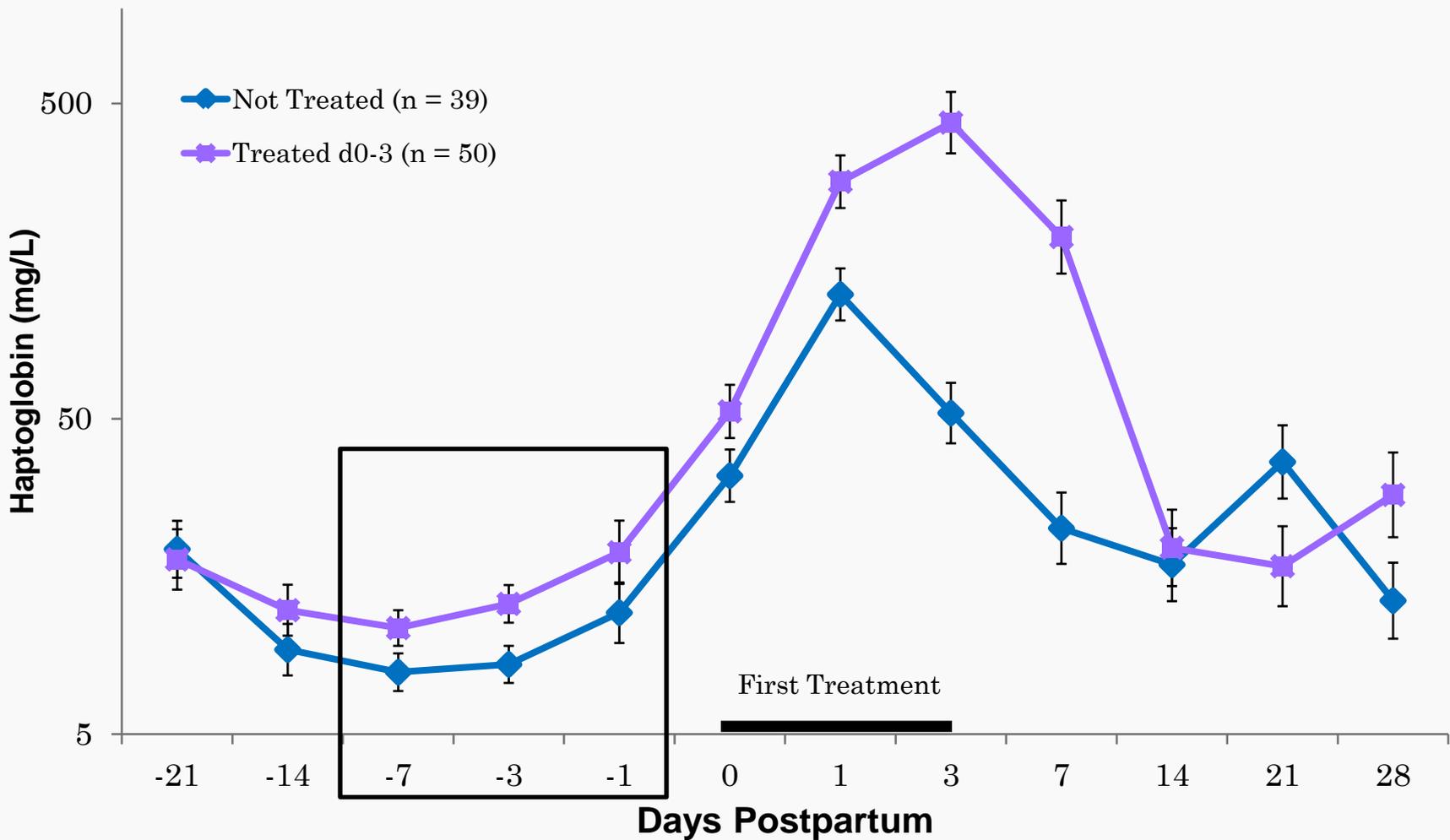


Figure 8: Cows that were treated first within day 0 to 3 postpartum had greater [Hp] at days -7 ($P = 0.05$) and -3 ($P = 0.01$) postpartum.

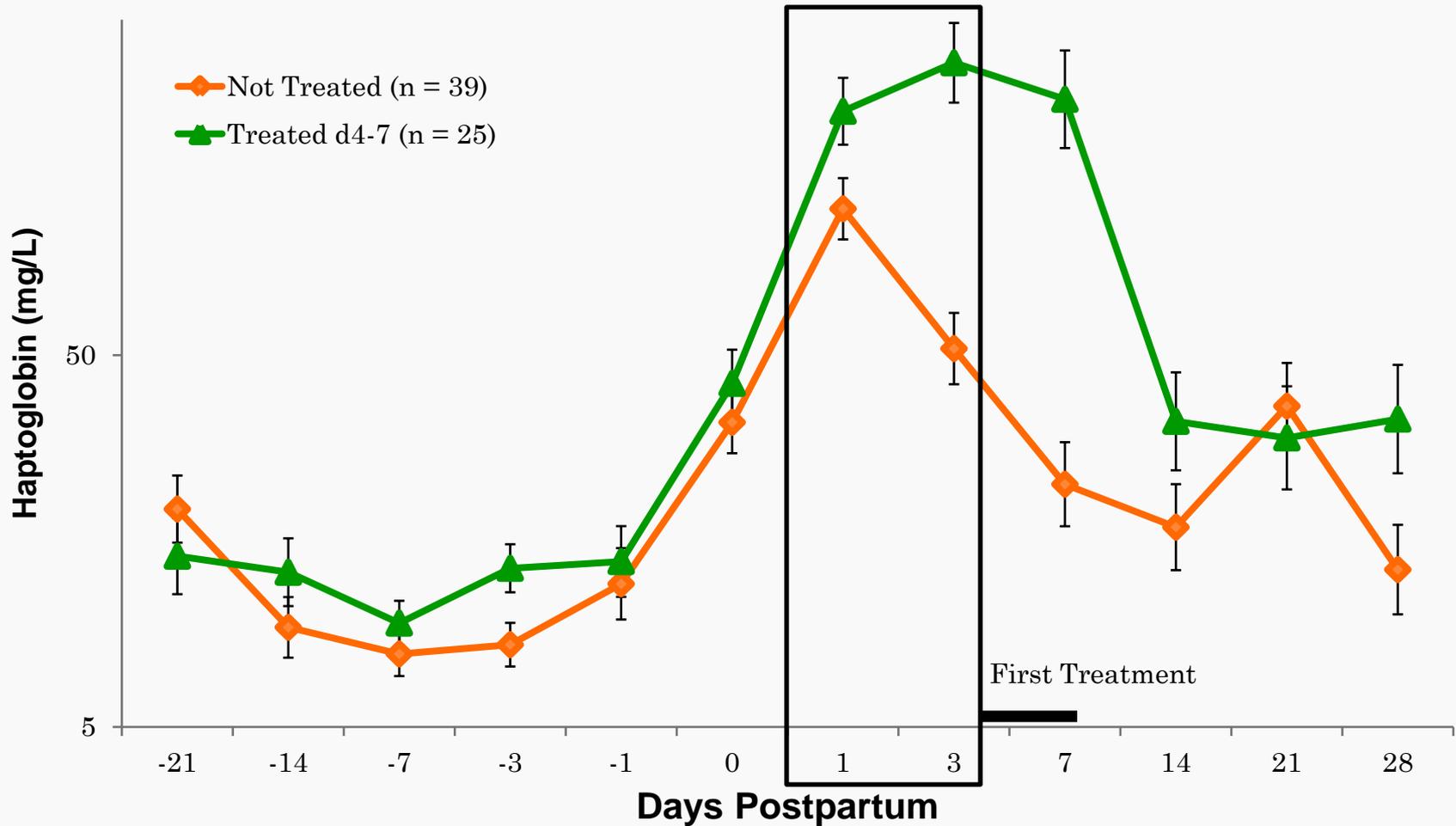


Figure 9: Cows that were treated first between 4 and 7 days postpartum had greater [Hp] at days 1 ($P = 0.04$) and 3 postpartum ($P < 0.001$).

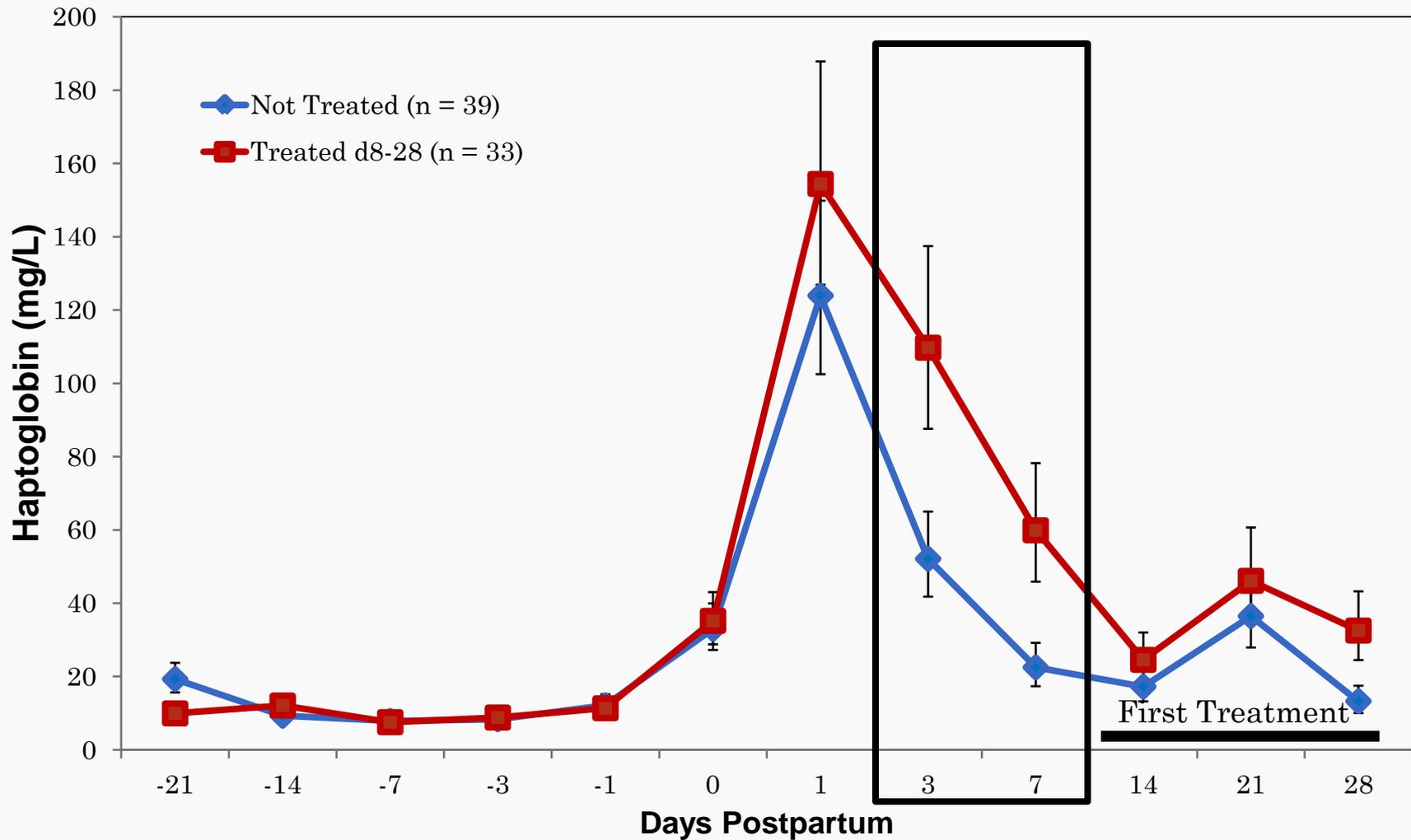


Figure 10: Cows that were treated first between 8 and 28 days postpartum had greater [Hp] at days 3 ($P = 0.002$) and 7 ($P < 0.001$) postpartum.

SUMMARY

- Elevated serum haptoglobin concentrations during first week postpartum indicate disease:
 - Incidence
 - Severity
 - Number
 - Type



SUMMARY

- Elevated serum haptoglobin concentrations precede birth complications and clinical diagnosis and treatment of peripartal diseases



CONCLUSION

- Serum haptoglobin may assist in early detection and treatment of diseases in early lactation



IMPACT

- Increased profit
- Shorter time period between parturition and resumption of estrus cycle
- Consistent dairy products for consumers
- Happy cows! 😊



FUTURE RESEARCH

- Repeat the study on a larger scale and at various farms that differ in management protocols
 - Include heifers
 - Diseases to be diagnosed by a veterinarian



Thank you:

- Dr. Gerd Bobe and my fellow laboratory peers
- Diamond V, ER Jackman Internship Support Program, and USDA's Multicultural Scholars Program
- Van Beek Dairy
- Family and friends!



QUESTIONS?



FUNCTIONS OF HAPTOGLOBIN

○ Antioxidant

- Binds to free-floating hemoglobin to prevent unwanted oxidation
- Transports complex to CD163 receptor on monocytes, then degraded in the lysosomes



FUNCTIONS OF HAPTOGLOBIN

○ Anti-Inflammatory Agent

- Prevents oxidation damage of cells, hence, the release of pro-inflammatory cytokines
- Inhibits the activity of cyclooxygenase (COX) and lipoxygenase (LOX) in platelet cells
 - COX and LOX promote inflammation and oxidation of LDL



FUNCTIONS OF HAPTOGLOBIN

- Tissue-Regeneration Agent
 - Promotes the migration of fibroblasts needed for tissue regeneration
 - Inhibits the activities of matrix metalloproteinases, which promote tissue breakdown



FUNCTIONS OF HAPTOGLOBIN

- Bacteriostat
 - Prevents the growth of pathogenic bacteria that require the iron from hemoglobin



FUNCTIONS OF HAPTOGLOBIN

○ Immunomodulator

- Attracts monocytes and macrophages to site of infection
 - Binds to decrease their production of pro-inflammatory cytokines

