# Disease Prevalence and Its Consequences on Blood Metabolites, Physical Activities, Milk Yield, and Fertility

*J.S. Stevenson and S. Banuelos*

## Summary
Health status of 160 lactating cows was monitored by assessing blood metabolites on days 0, 3, 7, and 14 after calving, in addition measures of physical activity during 20 days surrounding parturition. Cows with clinical disease (any with diagnosis of ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk) were compared with outcomes in healthy cows. Expected differences were observed between health status groups for serum concentrations of free fatty acids, beta-hydroxybutyrate, haptoglobin, and calcium, but not for plasma glucose. Daily postpartum rumination and eating times were decreased in diseased cows and they spent more time resting or being inactive. Body condition scores decreased more in diseased cows, whereas body weight and milk yield were unaffected by health status. Despite early and proportionally more ovulations during the prebreeding period in healthy cows, pregnancy rate at first service and days to conception were not affected by health status, likely because of good health care of all cows having both clinical and subclinical disease.

## Introduction
Approximately 50% of the dairy cows in the U.S. suffer from at least one disease event during the first 60 days in milk. Transition from pregnancy (no lactation) to lactation (not pregnant) presents the greatest risk for culling and death for a dairy cow. During this transition period, a number of metabolic and endocrine adaptations must occur to keep cows healthy.

Calving-related disorders and diseases that affect the reproductive tract are major contributors to poor fertility. In 2013, the most common clinical diseases in cows reported by dairy producers were mastitis (24.8%), any degree of lameness (16.8%), infertility (8.2%), and metritis (6.9%). Cows that have one of the aforementioned disorders were 50 to 63% less likely to resume estrous cycles by the end of the voluntary waiting period, and were 25 to 38% less likely to become pregnant after the first AI-breeding compared with healthy cows.

Relationships of health and metabolic markers, in addition to measures of resting, eating, rumination, and activity derived from CowSensor ear tags, have not been examined to determine their relationships to subsequent ovulation, estrus, pregnancy,

and health status. Our objective was to characterize: (1) various metabolic (free fatty acids [FFA], beta-hydroxybutyrate [BHB], glucose, haptoglobin, and calcium) and (2) physical (body condition score, body weight, eating, rumination, and activity times) traits that affect milk yield and reproductive performance of healthy and clinically diseased lactating cows.

## Experimental Procedures

Close-up nulliparous and dry Holstein cows (n = 160; Kansas State University Dairy Teaching and Research Center, Manhattan, KS) of mixed parity were enrolled between December 2017 and August 2018. Close-up cows enrolled in the study were housed in an open-front, straw-bedded maternity barn until parturition. Cows were housed in open lot free-stall barns bedded with sand after calving, and milked thrice daily. In addition to the proposed data collection, cows were monitored daily by herd personnel for body temperature and urine ketones during the first 10 days after calving to identify health disorders such as dystocia, retained placenta, mastitis, displaced abomasum, milk fever, and lameness. Any cow for which a clinical diagnosis of ketosis, metritis, mastitis, respiratory disease, or milk fever was made during the first 60 days in milk was classified as diseased, whereas the remaining cows were classified as healthy, even though they may have manifested some other clinical or subclinical disease.

### Metabolic Measures

Blood samples were collected on days 0, 3, 7, and 14 to assess serum concentrations of FFA (proxy for negative energy balance), BHB (proxy for ketosis), calcium (marker for hypocalcemia), and haptoglobin (proxy for general inflammation), and plasma concentrations of glucose (proxy for energy status; Figure 1). Body condition scores and body weights were assessed weekly beginning at calving until $73 \pm 3$ days in milk.

### Definitions and Assessment of Disease

- Retained placenta: failure to expel fetal membranes by 24 hours after calving.
- Metritis: evidence of brown watery exudate detected by palpation per rectum at 0, 4, 7, 10, and $14 \pm 3$ days in milk (day 0 = day of calving).
- Ketosis: concentration of BHB in plasma greater than 10 mg/dL on day 0, 3, 7, or 14 after calving.
- Dystocia: any cow with twins or a calving difficulty score of 3 or greater. Calving difficulty scores: 1 = no problem; 2 = slight problem; 3 = use of obstetrical chains; 4 = use of a calf jack.
- Displaced abomasum: diagnosis was made on history and clinical signs in combination with auscultation findings (distinct ping identified by using a stethoscope).
- Subclinical mastitis: somatic cell count exceeding 310,000 cells/mL during the first 60 days in milk.
- Clinical mastitis: any evidence of abnormal milk observed by milkers.
- Milk fever: downer cow with blood calcium concentrations < 7.5 mg/dL.

### Reproductive Traits

Blood samples also were collected weekly beginning $21 \pm 3$ until $73 \pm 3$ days in milk to determine the onset of luteal function by assessing progesterone concentration. Estrual events were recorded by CowSensor ear tags (Agis CowManager, the Netherlands) by

using accelerometer technology. Beginning at $63 \pm 3$ days in milk, cows were enrolled in a modified Ovsynch ovulation synchronization program (GnRH — 7 days — prostaglandin $F_{2a}$ — 24 hours — prostaglandin $F_{2a}$ — 32 hours — GnRH — 16 hours — AI) to facilitate first postpartum AI.

## Results and Discussion
### Clinical Disease Prevalence
Occurrences of clinical and subclinical disease are summarized in Table 1. By definition in our study, diseased cows were those diagnosed with ketosis, metritis, mastitis, respiratory disease, or milk fever. The remaining disease conditions—calving problems (including dystocia and retained placenta), subclinical mastitis, subclinical low blood calcium (hypocalcemia), lameness, and digestive issues (including off-feed, severe diarrhea, or displaced abomasum)—also were diagnosed more ($P < 0.05$) often in diseased cows compared with healthy cows. Prevalence of the diseases in Table 1 is consistent with what has been observed in other herds and in the scientific literature.

### Blood Metabolites
Blood metabolites were diagnostic of disease and consistent with expectations of unhealthy cows. Concentrations of FFA in blood serum were greater ($P < 0.05$) in diseased compared with healthy cows at calving and on days 3, 7, and 14 postpartum, suggesting greater body fat loss and greater negative energy balance in diseased cows (Figure 2; top panel).

Concentrations of BHB in blood serum, evidence of clinical and subclinical ketosis, were greater ($P < 0.05$) in diseased compared with healthy cows on days 3 and 14 postpartum, and tended ($P = 0.08$) to be greater on day 10 (Figure 2; second panel). Concentrations of haptoglobin in blood serum, an indirect measure of immune activation, were greater ($P < 0.05$) in diseased compared with healthy cows on days 0 and 3 (Figure 2; third panel).

Concentrations of calcium in blood serum were reduced ($P < 0.05$) in diseased compared with healthy cows on days 0 and 3, and tended ($P = 0.07$) to remain lower on days 10 and 14 (Figure 2; bottom panel). Rectal temperatures (°C) tended ($P = 0.09$) to be slightly greater in diseased than healthy cows on day 3 ($39.0 \pm 0.05$ vs. $38.8 \pm 0.07$), but did not differ on day 7 ($39.1 \pm 0.04$ vs. $39.0 \pm 0.05$). Blood plasma glucose, a proxy for energy status, was not affected by health status on days 0, 3, 7, and 14 after calving.

### Physical Activity
Physical traits of rumination, resting, eating, ear skin temperature, and general activity assessed by the CowSensor ear tags were analyzed for the periods 10 days before and 10 days after calving. Cows subsequently diagnosed as diseased had no observable changes in physical traits assessed during the prepartum period. Rumination time decreased acutely from day -2 until day 2 relative to calving (Figure 3; top panel) in all cows, whereas eating time decreased gradually during the last 10 days of gestation (Figure 3; bottom panel). Resting activity during 10 days before calving was constant (Figure 3; middle panel), whereas general activity or high activity increased acutely

24 hours before calving and reached a peak between 24 and 48 hours after calving (not shown) before decreasing.

Significant changes in rumination and resting were observed shortly before and after calving and differed according to health status. Healthy cows had greater ($P < 0.01$) rumination and eating times during the first 10 days after calving and rested less or were more ($P < 0.01$) active than diseased cows. Greater resting or inactive times were consistent with a less healthy state of the cows.

Daily minimum and maximum ear skin temperatures assessed by the ear tags before and after calving did not differ between healthy and diseased cows but were highly correlated (r = 0.91 to 0.95; $P < 0.001$) with environmental temperatures.

### Milk and Body Traits

Although average body weight did not differ between healthy and diseased cows during the prebreeding period (Table 2), body condition score was reduced in diseased cows (Table 2; Figure 4). Loss of body condition, which averaged 3.0 ± 0.33 at calving, was more rapid in diseased than healthy cows and remained less throughout the entire prebreeding period. Healthy cows started to increase in body condition after 7 weeks postpartum. Despite these differences, milk yield was not different between health status groups (Table 2).

### Reproductive Traits

Healthy cows ovulated sooner ($P < 0.01$) after calving and tended ($P = 0.13$) to express their first estrus earlier than diseased cows (Table 3). Duration of first estrus also tended ($P = 0.12$) to be longer in healthy than diseased cows. Healthy cows had proportionally more ($P < 0.01$) ovulations and tended ($P = 0.07$) to have more estrus periods before first insemination. Pregnancy at first insemination and days to conception were not affected by health status (Table 3).

Direct effects of clinical disease on reproductive traits have been reported. Eggs collected from cows with at least one case of clinical disease had reduced fertilization rates and embryos had compromised quality as early as 5 to 6 days after AI. When conceptuses (embryos and their developing placenta) were collected on day 15 after AI from 145 lactating dairy cows in a second study, fewer cows with clinical diseases were pregnant. When one clinical disease was diagnosed during the first 60 days in the latter study, percentage of cows pregnant on day 15 after AI decreased from 49.4 to 29.8% and uterine disease reduced the percentage of cows pregnant by more than half (49.4 to 20%).

In another study, cows that maintained or gained body condition (n = 226) between calving and the onset of the Ovsynch programs had greater pregnancy rates (41.6 vs. 32.3%) than those that lost body condition (n = 170). Cows with body condition scores ≤ 2.5 at timed AI had poorer pregnancy rates than cows with greater body condition, but the negative impact on pregnancy rate was greater for cows that lost body condition during the first 3 weeks postpartum compared with those that either maintained or gained body condition.

## Conclusions

Elevated FFA, BHB, and haptoglobin, and reduced calcium were detected in cows with clinical disease, reflecting greater negative energy balance, more ketosis, altered immune function, and more hypocalcemia compared with healthy cows. Daily postpartum rumination and eating times were greater for healthy than diseased cows, which spent less time resting or inactive than diseased cows. Body condition scores were poorer in diseased cows, whereas body weight and milk yield were unaffected by health status. Despite early and proportionally more ovulations during the prebreeding period, pregnancy rate at first service and days to conception were not affected by health status, likely because of good health care of all cows having both clinical and subclinical disease.

**Table 1. Disease incidence in 160 lactating dairy cows calving during December 2017 and August 2018**

| Item | Health status | | |
| --- | --- | --- | --- |
| | Healthy, % | Clinical disease,[1] % | P-value |
| Number of cows | 104 | 56 | |
| Ketosis[2] | 0.0 | 58.5 | <0.01 |
| Metritis[3] | 0.0 | 28.3 | <0.01 |
| Clinical mastitis[4] | 0.0 | 42.5 | <0.01 |
| Milk fever | 0.0 | 2.83 | 0.02 |
| Calving problems[5] | 1.89 | 8.5 | <0.01 |
| Subclinical mastitis[6] | 24.1 | 32.1 | 0.04 |
| Subclinical hypocalcemia[7] | 43.4 | 60.4 | 0.04 |
| Lameness | 0.0 | 4.7 | <0.01 |
| Digestive | 0.0 | 26.4 | <0.01 |

[1]Any cow with diagnosed ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk.
[2]Based on beta-hydroxybutyrate concentrations > 10 mg/dL on day 0, 3, 7, or 14 after calving.
[3]Diagnosed by evidence of brown watery exudate detected by transrectal palpation at 0, 3, 7, and 14 days in milk (day 0 = day of calving).
[4]Any case of abnormal milk.
[5]Any cow with twins or a calving difficulty score of 3 or greater.
[6]Based on any incidence of somatic cell counts that exceed the threshold 310,000 cells/mL during the first 60 days in milk.
[7]Based on calcium concentrations < 8.6 mg/dL on day 0, 3, 7, or 14 postpartum.

**Table 2. Body score, body weight, and milk yield in healthy and diseased lactating dairy cows calving between December 2017 and August 2018**

| Item | Health status | | |
| --- | --- | --- | --- |
| | Healthy | Clinical disease[1] | P-value |
| Body condition score[2] | 2.6 ± 0.03 | 2.5 ± 0.03 | 0.02 |
| Body weight,[2] lb | 1,559 ± 9.5 | 1,541 ± 6.8 | 0.34 |
| 305-day energy-corrected milk, lb | 26,893 ± 195 | 26,699 ± 137 | 0.71 |
| Cumulative 14-week milk, lb | 4,412 ± 97 | 4,439 ± 71 | 0.82 |
| Mean 14-week daily milk, lb/day | 101.6 ± 0.9 | 100.1 ± 0.6 | 0.56 |

[1]Any cow with diagnosed ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk.
[2]Mean of measures from 1 week post-calving until AI at 73 ± 3 days in milk.

**Table 3. Reproductive characteristics of healthy and diseased dairy cows**

| Item | Health status | | |
| --- | --- | --- | --- |
| | Healthy | Clinical disease[1] | P-value |
| Days to first ovulation | $31.6 \pm 2.1$ | $39.7 \pm 1.5$ | 0.01 |
| Days to first estrus | $40.8 \pm 3.0$ | $46.7 \pm 2.5$ | 0.13 |
| Duration of first estrus, hours | $15.8 \pm 1.8$ | $12.1 \pm 1.5$ | 0.12 |
| Peak first estrus activity, arbitrary units | $5.2 \pm 0.3$ | $4.8 \pm 0.3$ | 0.32 |
| Cows with one or more heats before timed AI, % | 72.2 | 57.5 | 0.07 |
| Cows with one or more ovulations before timed AI, % | 85.2 | 65.1 | 0.01 |
| Days to conception | $146 \pm 11$ | $151 \pm 8$ | 0.65 |
| Pregnant to first service,[2] % | 34.6 | 30.0 | 0.58 |

[1]Any cow with diagnosed ketosis, metritis, mastitis, respiratory disease, or milk fever during the first 60 days in milk.

[2]Ovulation was synchronized before timed AI (GnRH — 7 days — prostaglandin $F_{2a}$ — 24 hours — prostaglandin $F_{2a}$ — 32 hours — GnRH — 16 hours — AI).
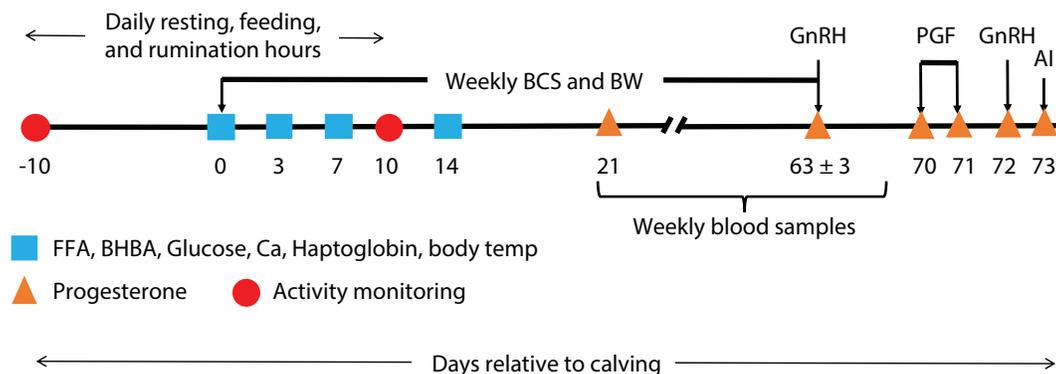


**Figure 1. Experimental scheme. Body temperature and blood samples were collected on days 0, 3, 7, and 14 after calving to assess concentrations of free fatty acids (FFA), beta-hydroxybutyrate (BHBA), glucose, calcium (Ca), and haptoglobin. Weekly body scores and blood were collected weekly starting at day 21 postpartum through time AI on $73 \pm 3$ days in milk.**
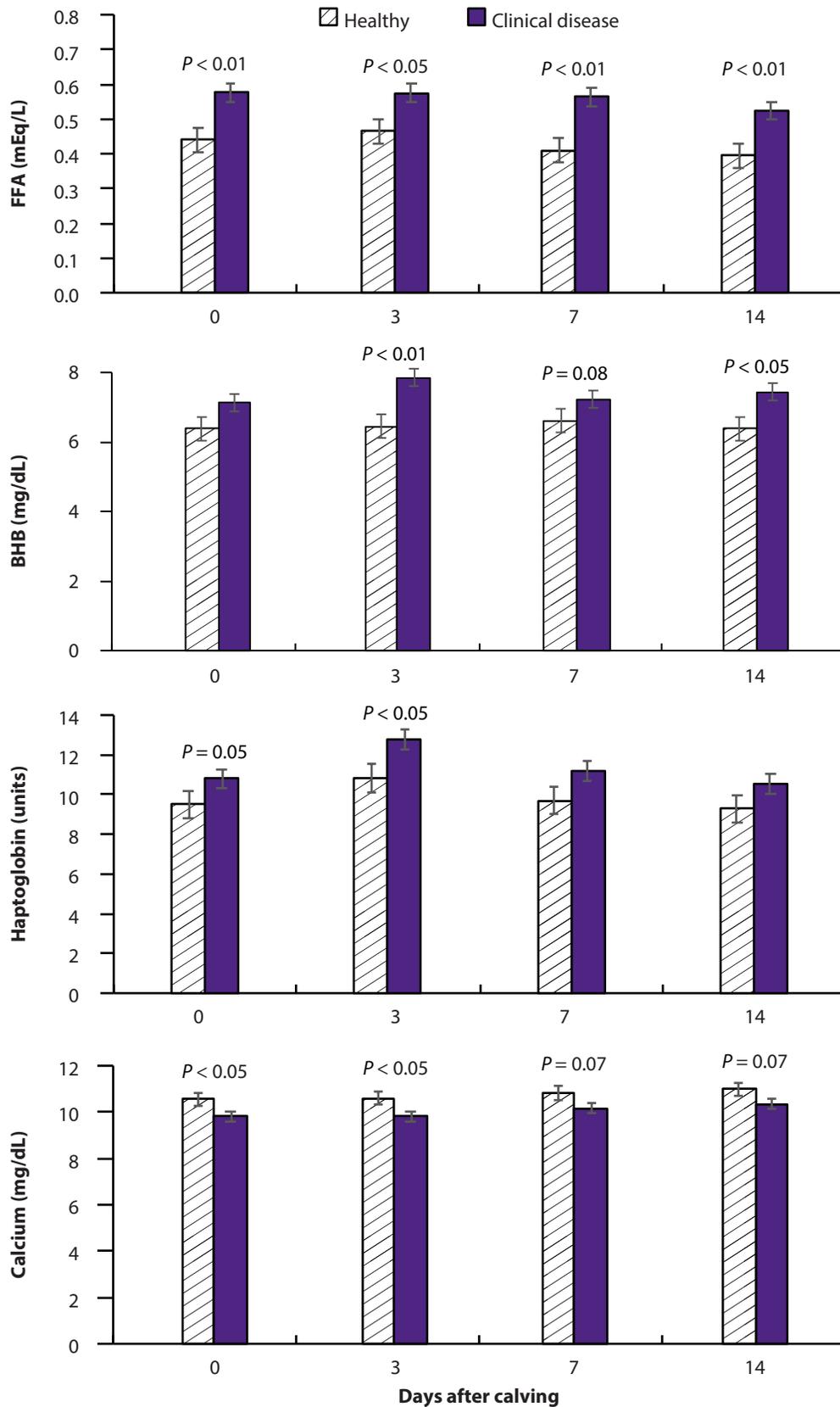
Figure 2. Plasma concentrations of free fatty acids (FFA; top panel), beta-hydroxy butyrate (BHB; second panel), haptoglobin (third panel), and calcium (bottom panel) in 160 dairy cows (calving from December 2017 through August 2018) on days 0, 3, 7, and 14 after calving in 54 healthy cows and 106 cows diagnosed with clinical disease (i.e., ketosis, metritis, mastitis, respiratory disease, or milk fever).
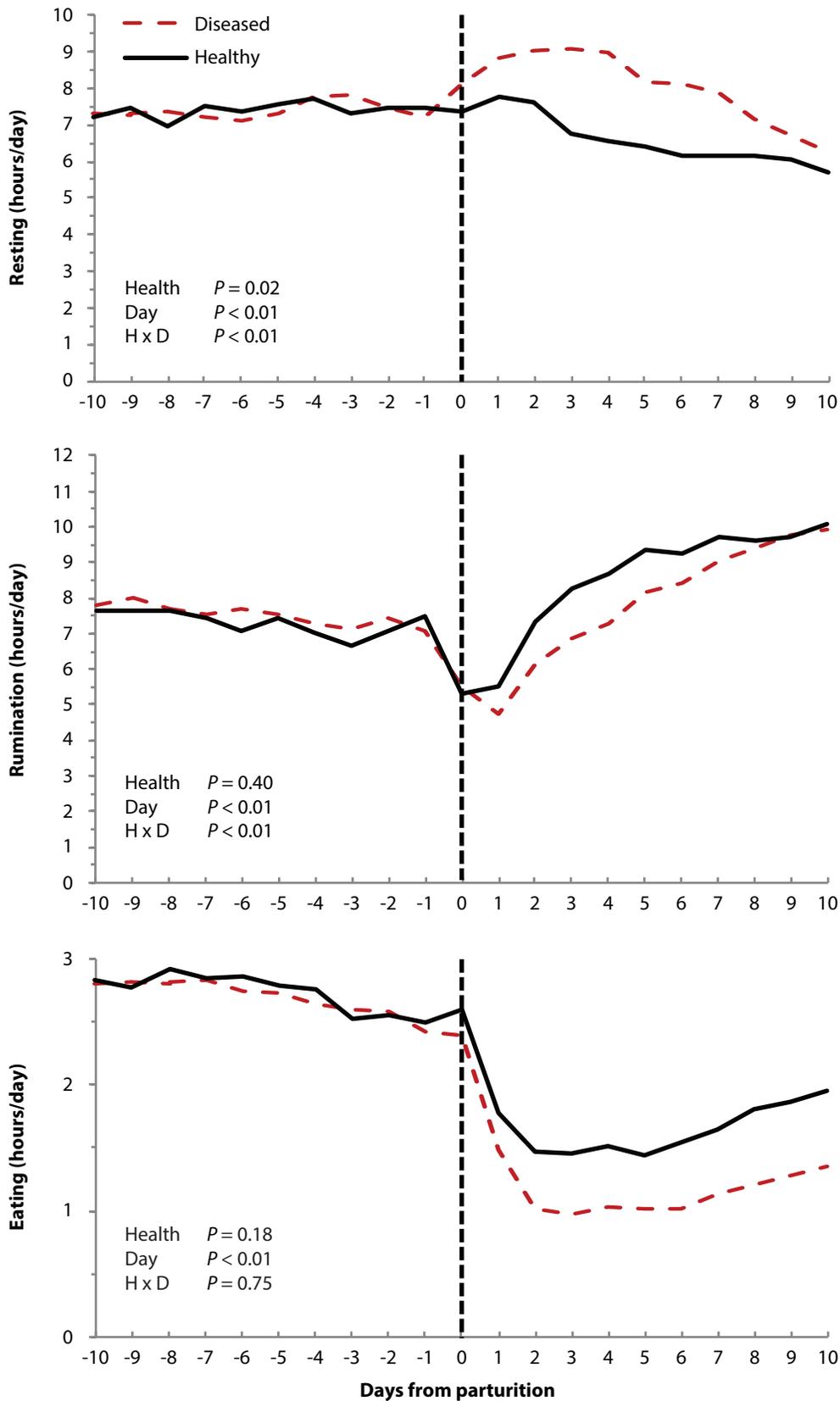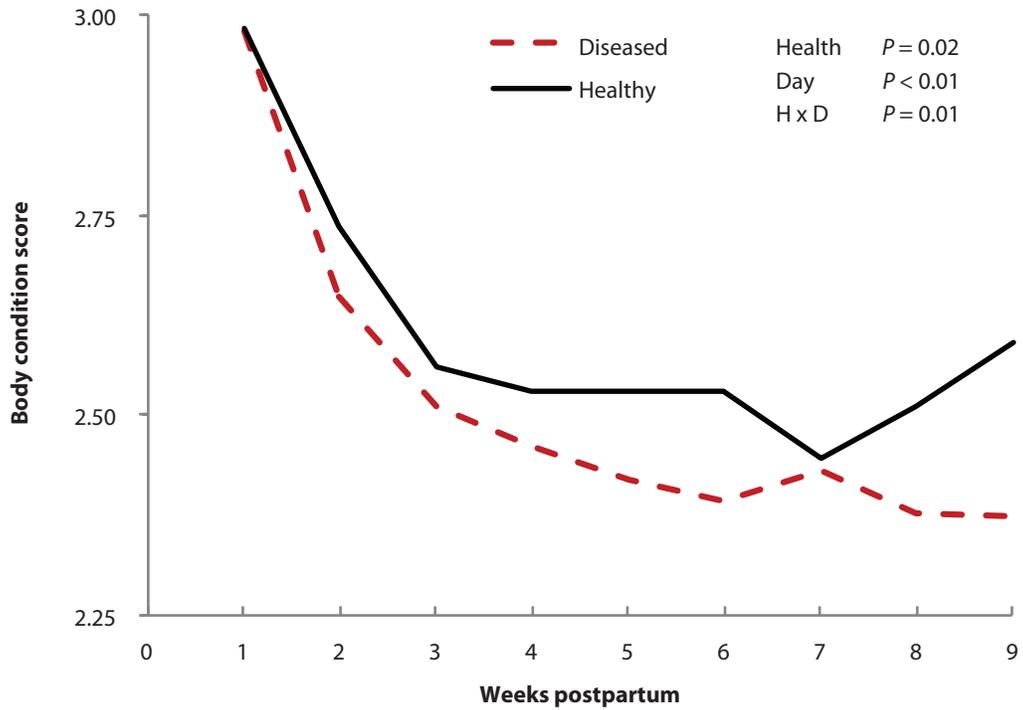
Figure 3. Resting (upper panel), rumination (middle panel), and eating (lower panel) hours per day in 160 dairy cows (calving from December 2017 through August 2018) on day -10 through day +10 from parturition in 54 healthy cows and 106 cows diagnosed with clinical disease (i.e., ketosis, metritis, mastitis, respiratory disease, or milk fever).

Figure 4. Changes in body condition score during the prebreeding period after calving in 54 healthy cows and 106 cows diagnosed with clinical disease (i.e., ketosis, metritis, mastitis, respiratory disease, or milk fever).