Combined Risk Factors and Digestive Disorders in Mid-Lactation Holstein Cows: A Case Study

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Summary
Digestive disorders can be a significant cause of disease on dairies and are frustrating because of their unpredictability. Diets that may support excellent health in most cases may nonetheless result in significant gastrointestinal disease, even leading to deadly conditions such as hemorrhagic bowel syndrome. To our knowledge, there is limited research on these conditions, as many risk factors fail to reproduce disease when experimentally administered to cows, leading many to conclude that these disorders are generally multifactorial in nature and difficult to replicate. In this case study, we document the outbreak and resolution of digestive disorders among 15 control cows enrolled in a larger production study. Over 14 weeks, cows were individually fed, with milk yield and composition, blood variables, and health observations recorded. The diet included drought-stressed corn silage that introduced difficulties including low energy density, high dry matter content (making it unstable at feedout), and mycotoxin contamination. By weeks 4–5 on the study, sporadic diarrhea began to appear and milk fat content had dropped from 3.7% to 3.4%, on average. Coincident with the onset of environmental heat stress, three cows developed severe digestive disorders, resulting in a displaced abomasum in one cow. At that point, the diet was changed to replace some corn silage with wheat straw, a direct-fed microbial was added to the diet, and organic acid treatment of the silage face was initiated. Within a month after these changes were implemented, essentially all signs of digestive problems resolved, including milk fat content, fecal consistency, and blood plasma concentrations of haptoglobin and D-lactate. This case study points to multiple factors that likely combined to lead to microbial and gastrointestinal disruptions resulting in clinical disease in a subset of cows.

Introduction
Digestive disorders, ranging from mild diarrhea to hemorrhagic bowel syndrome, occur among lactating cows on many farms. These challenges can happen sporadically, often leaving producers and their advisors frustrated by the lack of any obvious cause of the problem. A wide variety of factors are thought to contribute to these digestive challenges, including the presence of opportunistic gastrointestinal pathogens, excessive flow of fermentable carbohydrate or protein to the hind gut, and the presence of mycotoxins in the diet. These conditions are thought to contribute to a disruption of

1 Biomin America Inc., Overland Park, KS.
the gut barrier (i.e., “leaky gut”), which in turn leads to bacterial invasion and dramatic inflammation of the tissue. However, research progress on this topic has been slow, as challenge studies that have introduced individual risk factors have generally failed to replicate the digestive disorders.

On-farm outbreaks of gastrointestinal disease probably differ from controlled studies in that multiple risk factors occur simultaneously. If these factors could be documented over the course of such an outbreak, it could help to improve our understanding of the disease process. Unfortunately, commercial farms almost never have relevant samples collected prior to this kind of outbreak, preventing analysis of factors contributing to onset of the disease.

In the process of conducting a study, we recently dealt with an outbreak of gastrointestinal disease. Fortunately, we collected samples for the purpose of the primary study that allowed us to document some relevant changes that likely contributed to disease progression and resolution over the course of 14 weeks. Our objective in this case study is to describe our observations and evaluate plausible explanations for the disease process.

**Experimental Procedures**
Fifteen multiparous Holstein dairy cows (10 in lactation 2, 4 in lactation 3, and 1 in lactation 4) between 94–197 days in milk were housed at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS) in tie-stalls with rubber mats and wood shavings. These cows were part of a larger production study; here we are reporting responses in control cows only. Cows were fed a total mixed ration (TMR; Table 1) twice daily, consisting of corn silage, alfalfa hay, corn milling product (Sweet Bran, Cargill, Blair, NE), whole cottonseed, and a grain mix. The corn silage was from 2018 and was drought-stressed—as a result, it had low starch content, which required us to add more concentrate to meet energy requirements. Feed and feed refusal samples were collected every week, combined into bi-weekly composites, and analyzed for chemical composition.

Cows were milked 3 times daily at 04:00, 11:00, and 16:00 hours. Milk yield was recorded at each milking and milk samples were collected for 6 consecutive milkings on Thursday and Friday every week for component analysis. In addition, blood samples were collected from the tail vein once every other week between the second and third milkings. Plasma was collected and later analyzed for intestinal health biomarkers.

Data were analyzed to assess the effects of study week, parity, and their interaction, while accounting for the random effect of cow in a repeated-measures statistical model.

**Results and Discussion**
Over the course of the study, 5 of the 15 cows were removed from the study over health concerns. Some loose manure was noted relatively early in the study, particularly from week 4 on; however, for most cows and most days, manure consistency was within the normal range.
The first cow to be removed from study was in her fourth lactation and developed hock inflammation in week 4. The next three cows were removed from the study for digestive disorders. During week 5, the first serious summer heat stress window occurred, with mean weekly environmental temperature-humidity index climbing past 70 (Figure 1). At the end of week 5, a second-lactation cow suddenly (within 24 hours) went off feed and stopped producing milk. Physical examination revealed extremely high rumen motility, but her body temperature and water intake were normal. Within 48 hours, she was diagnosed with a displaced abomasum. In week 7, two more second-lactation cows were removed from the study within 48 hours of each other after 4 days of declining feed intake. Digestive tract abnormalities (high motility, diarrhea) were again observed, and one of these cows also showed some apparent neurological issues.

At this point in the study, samples were sent for initial mycotoxin analysis and the diet was adjusted to partially replace an excessively dry corn silage (>40% dry matter) with a more typical corn silage and some straw to enhance effective fiber content of the diet. Furthermore, we began treating the silage faces with organic acids (Ultra-Curb, Kemin, Des Moines, IA) to limit mold growth at feed-out and added an anti-mycotoxin product (Biofix Plus Pro; Biomin America, Overland Park, KS) to the grain mix at 0.10% of the ration (dry matter basis). All of these changes were in place by the end of week 8. One additional cow (third lactation) was removed from the study during week 13 due to clinical mastitis.

Dry matter intake (Figure 2) was greater for cows in parity 3+ vs. parity 2 ($P = 0.01$), and it varied by week ($P < 0.01$), with weeks 2 and 4 differing from week 1 ($P < 0.05$). The uptick in feed consumption in week 2 likely reflects adaptation to the new diet, whereas shifts up and down after that time likely reflect a combination of responses to heat stress and declining milk yield with advancing days in milk.

Milk yield is shown in Figure 3. Not surprisingly, parity 3+ cows produced more milk than parity 2 cows ($P = 0.05$). In addition, milk yield varied by week ($P < 0.01$) with lower production in weeks 2, 3, 9, 10, 11, 12, 13, and 14 relative to week 1 ($P < 0.05$). Although a progressive decrease in milk yield is expected in this group of cows past peak lactation, the more dramatic decline in milk yield from weeks 9 through 14 was likely a response to the change in diet, particularly the inclusion of dietary straw to increase effective fiber content. Previous research has shown that adding slowly-fermenting fiber to lactation diets typically decreases fluid milk yield.

Parity groups did not differ in milk fat content ($P = 0.47$, Figure 4); however, there was week-to-week variation ($P = 0.01$). Fat content was decreased in weeks 2, 3, 4, 5, 7, 9, and 10 relative to week 1 ($P < 0.05$) before recovering in weeks 11–14. Second lactation cows tended to have greater milk protein content compared with older cows ($P = 0.098$, Figure 5). In addition, weeks 2, 4, 7, 8, 10, 11, 13, and 14 all differed from week 1 ($P < 0.05$). Energy-corrected milk yield was greater in parity 3+ cows vs. second lactation cows ($P = 0.02$; Figure 6), and weeks 2, 9, 10, 11, 12, 13, and 14 were different from week 1 ($P < 0.05$). Feed efficiency (defined as ECM yield /dry matter intake) is shown in Figure 7. Cows in lactation 3+ lactations group had greater FE than younger cows ($P = 0.01$), and efficiency was greater in weeks 2 and 4 vs. week 1 ($P < 0.05$).
Blood samples collected biweekly throughout the study were analyzed for haptoglobin, an acute phase protein that is elevated during systemic inflammation. Plasma haptoglobin concentrations (Figure 8) tended to be greater in second lactation cows vs. parity 3+ cows ($P = 0.08$), and week 1 differed from weeks 3, 5, 9, and 11 ($P < 0.05$), reflecting a rise above baseline in the middle of the study and a significant decrease by week 11. This temporal pattern aligned with visual observations of digestive function (e.g., gut motility, fecal consistency) and suggested that changes put in place in weeks 7–8 likely had a positive impact on the inflammatory status of cows by week 11. D-lactate concentrations (Figure 9) in plasma tended ($P = 0.051$) to be greater in second lactation cows compared to parity 3+ cows, and weeks 7, 11, and 13 differed from week 1 ($P < 0.05$). Like haptoglobin, D-lactate rose gradually from week 1, peaking in week 7 and then declining to concentrations less than baseline by weeks 11–13. As D-lactate is produced primarily by microbial metabolism, its increased concentration in plasma by week 7 likely indicates a decline in gut barrier function (leaky gut) and/or excessive hind-gut fermentation, with an apparent return to a more normal status by the end of the study. It is also notable that all 3 of the cows that left the study due to digestive disorders were second-lactation cows.

Twice during the study (weeks 9 and 14), feed and fecal samples were collected to enumerate viable clostridia bacteria and those from the species *C. perfringens* specifically. The drought-stressed dry corn silage had clostridia concentrations in week 14 almost 50× greater than samples collected in week 9, and the concentration of clostridia in the TMR increased from weeks 9 to 14 (Table 2). In contrast, fecal samples showed decreased total clostridia as well as *C. perfringens* from weeks 9 to 14, suggesting a shift in the gut microbial ecosystem that inhibited this population. This disparity could be the result of the diet composition change, or potentially a change in mycotoxin-microbe-gut interactions following the incorporation of mitigation strategies.

Mycotoxin concentrations detectable in the ration throughout the study are represented in Figure 10. There is relatively little evidence available to establish concentrations of mycotoxins that are of concern for ruminants. There is some information regarding responses to aflatoxins, but we did not detect that class in any ration samples analyzed. Instead, we found that cows were constantly exposed to type B trichothecenes at diet concentrations between 843 and 1,069 parts per billion (ppb, as-fed). *Fusarium* fungi produce trichothecenes including nivalenol and deoxynivalenol (DON, also known as vomitoxin), the two compounds detected in our samples. Zearalenone is also produced by *Fusarium* fungi, but is an estrogenic metabolite. During the study, zearalenone concentrations in ration samples hovered around the detection limit of 51.7 ppb (as-fed), with a peak concentration of 109.8 ppb detected. On their own, these mycotoxins present challenges to producers, but previous research has shown that exposure to mixtures of mycotoxins can have a more acute impact.

As a case study, interpretation of these observations must be carried out cautiously. Multiple factors were changing simultaneously during the course of the study, including weather conditions, microbial and mycotoxin contaminants, forage sources, and mitigation strategies. Furthermore, we must acknowledge that the removal of the most susceptible cows in the middle of the study likely contributed to several measurements returning to normal ranges by the end of the study.
With those caveats in mind, we propose that the gastrointestinal health challenges observed during this study emerged as a result of “stacked stressors.” The diet formulation likely introduced one risk factor; although not extreme, the diet was marginal in terms of supply of physically-effective fiber, which made disturbance of the gut microbial ecosystem more likely. Secondly, the consistent exposure to mycotoxins likely contributed to disruption of both the microbial populations and the gut itself. Finally, the onset of summer heat stress seemed to tip some of the cows over the edge into clinical disease.

Assuming that the proposed etiology is correct, what could have been done to avoid these problems? Because we did not see a drop in the measurable mycotoxins during the study (Figure 10) and heat stress continued to challenge the cows (Figure 1), the resolution of disease biomarkers and clinical signs of digestive problems by week 11–12 suggest that at least some of our changes were likely effective. Increasing dietary forage has a protective role in microbial stability in the gut, and directly contributes to slowing passage of feed through the gastrointestinal tract. Organic acid treatment of the silage face (particularly for an excessively dry silage) can help to limit fungal growth at feed-out, and mycotoxin binding products are effective at binding at least some mycotoxins, helping to wash them out of the gut with less impact. Unfortunately, due to more extreme weather patterns in the Midwest, mycotoxins are becoming more prevalent in animal feeding programs and need to be addressed.

In summary, we documented a clear outbreak of gastrointestinal disruption in mid-lactation cows, occurring with a mild milk fat depression and a significant increase in markers of systemic inflammation and gut microbial disruption. These issues resolved following dietary changes, although complete resolution of the problem took approximately a month. This case study provides insights into the disease process and ideas for dealing with similar problems on dairy farms.
### Table 1. Ingredient and chemical composition of total mixed ration (% dry matter (DM))

| Ingredients                        | Weeks 1–8 | Weeks 9–14 |
|------------------------------------|-----------|------------|
| Corn silage                        | 16.66     | 12.57      |
| Alfalfa hay                        | 20.78     | 20.93      |
| Corn milling product\(^1\)         | 17.36     | 17.48      |
| Cottonseed                         | 2.79      | 2.81       |
| Corn grain                         | 24.79     | 24.96      |
| Expeller soybean meal\(^2\)        | 12.15     | 12.23      |
| Limestone                          | 1.25      | 1.26       |
| Sodium bicarbonate                 | 0.85      | 0.86       |
| Calcium salts of long-chain fatty acids\(^3\) | 0.83 | 0.84 |
| Micronutrient pre-mix              | 2.54      | 2.44       |
| Wheat straw                        | -         | 3.51       |
| Direct-fed microbial\(^4\)         | -         | 0.11       |

| Nutrients                          |           |            |
|------------------------------------|-----------|------------|
| DM, %                              | 61.7      | 59.9       |
| Net energy for lactation, Mcal/kg DM | 1.69    | 1.63       |
| Crude protein                      | 18.5      | 19.4       |
| Ether extract                      | 5.03      | 5.01       |
| Neutral detergent fiber            | 31.4      | 31.3       |
| Acid detergent fiber               | 20.3      | 20.9       |
| Ash                                | 8.46      | 9.72       |
| Ca                                 | 1.00      | 1.25       |
| P                                  | 0.49      | 0.50       |

\(^1\)Sweet Bran, Cargill, Blair, NE.
\(^2\)Soy Plus, Landus Cooperative, Ames, IA.
\(^3\)Essentiom, Arm & Hammer Animal Nutrition, Princeton, NJ.
\(^4\)Biofix Plus Pro; Biomin America, Overland Park, KS.

### Table 2. Average clostridia and \(C. \) perfringens enumerated in feed and fecal samples

|                   | Clostridia CFU\(^1\)/g | \(C. \) Perfringens CFU/g |
|-------------------|------------------------|---------------------------|
|                   | Week 9 | Week 14 | Week 9 | Week 14 |
| Feed samples      |        |        |        |        |
| Total mixed ration| 1,280  | 8,700  | 100    | 50     |
| Dry corn silage   | 440    | 21,850 | 20     | 50     |
| Wet corn silage   | 20     | <10    | <10    | <10    |
| Fecal samples     | 34,000 | 5,000  | 31,000 | 4,100  |

\(^1\)Colonies forming units; an approximation of viable bacteria.
Figure 1. Averages for external maximum daily temperature (MaxTemp, °F), minimum daily temperature (MinTemp, °F), relative humidity (RH, %), and temperature-humidity index (THI) by week of the study.

Figure 2. Dry matter intake of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.
Figure 3. Milk yield of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ P < 0.05 vs. week 1.

Figure 4. Fat content of milk from cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ P < 0.05 vs. week 1.
Figure 5. Protein content of milk from cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
† P < 0.05 vs. week 1.

Figure 6. Energy-corrected milk yield of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
† P < 0.05 vs. week 1.
Figure 7. Feed efficiency of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

Figure 8. Blood plasma haptoglobin concentrations of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.
Figure 9. Blood plasma D-lactate concentrations of cows fed a ration naturally contaminated with mycotoxins. Values are means ± standard errors.
‡ $P < 0.05$ vs. week 1.

Figure 10. Mycotoxin concentration of composite ration samples (as-fed basis). Values are means, with trichothecenes being the summation of nivalenol and deoxynivalenol concentrations.
**Weeks where zearalenone concentrations were below detectable limit of 51.7 ppb.