Iowa State Beef Checkoff Research Program

Project title: The impact of high liver copper on BRD and vaccine responses in dairy × beef calves

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I. Nontechnical Summary:

Beef on dairy cattle are a growing segment of the beef industry, but their integration into feedlots remains a challenge to producers due in part to their heightened susceptibility to respiratory disease. Not only is respiratory disease costly to treat and prevent, but high-risk cattle such as young beef on dairy calves present an even greater economic challenge as they are twice as likely to succumb to respiratory disease. Previous research has shown dairy × beef cattle are exposed to greater amounts of copper (**Cu**) than native beef cattle due to over supplementation in the dairy industry. While Cu is a critical micronutrient essential for many processes, excess Cu may contribute to inflammation and immune dysregulation. We hypothesized that the increased Cu these calves are exposed to is a contributor to their heightened susceptibility to disease. To test this, sixty-four dairy × beef crossbred steers (209 lb, ~8 wk old) from multiple producers, but a single grower, and were blocked by body weight into 8-head pens upon arrival at the Iowa State University Beef Nutrition Farm. Steers were randomly assigned to one of two treatments: adequate liver Cu (**ADE**) or high liver Cu (**HIGH**), with 32 steers per treatment. After a preconditioning period to achieve desired liver Cu statuses, liver biopsies were collected to confirm Cu status and steers were assigned to Experiment 1 or Experiment 2. **Experiment 1:** Twenty-eight steers (n = 13 for ADE and n = 15 for high) were enrolled in a 49-d vaccine study. Liver Cu averaged 291 and 665 mg Cu/kg liver DM for ADE and HIGH, respectively. A respiratory vaccine (Bovilis Vista 5, Merck Animal Health, Madison, NJ) and an ovalbumin vaccine were administered on d 0, and boosters on d 21. Serum was collected on d 0 (initial vaccine), d 21 (booster), and d 49 to end the study and antibody titers in response to respiratory vaccination were measured. Serum was also used to determine haptoglobin concentrations, a marker of inflammation, and antibody production in response to ovalbumin. Both treatments responded equally to BRSV, BHV1, and BVDV2; however, neither treatment responded to BVDV1, likely due to previous viral exposure. There were also no differences detected in serum haptoglobin concentrations. HIGH steers had a greater initial response to the ovalbumin vaccine on d 21, but by d 49, ADE steers had similar antibody production, indicating the HIGH steers responded more quickly to the vaccine. This could potentially be due to increased inflammation in the HIGH treatment which primed the immune system to produce antibodies more rapidly than ADE. **Experiment 2:** Twenty-six steers were enrolled in a 13-d bovine respiratory disease challenge (n = 13 for ADE and n = 13 HIGH). Liver Cu averaged 279 and 603 mg Cu/kg liver DM for ADE and HIGH, respectively. Steers were trucked for 6 h and delivered to the Animal Resource Station in Ames, IA on d -1. Steers were infected with BRSV on d 0 and *Manheimia haemolytica* on d 5. Various measures were collected throughout disease challenge, including rectal temperature, clinical scores, plasma trace mineral status, viral and bacterial load, serum haptoglobin, total antioxidant capacity, and markers of liver damage. HIGH steers experienced a more severe increase in clinical disease scores than ADE steers. HIGH steers also tended to have a greater degree of damage and consolidation in the lung along with increased expression of NRF2, a gene involved in antioxidant status. Further, changes in plasma trace mineral concentrations over the course of infection suggest HIGH steers had an ablated nutritional immunity response. This suggests excess Cu resulted in heightened severity of respiratory disease, despite the fact that haptoglobin and total antioxidant capacity did not differ between treatments. In conclusion, excess liver Cu did not worsen vaccine response, but did cause increased severity of disease. Producers should avoid over-supplementing Cu, especially to dairy × beef steers, which may be more prone to inflammation.

a. Impacts

Excess liver copper did not impact overall antibody production in response to a respiratory vaccine, but it did result in faster antibody production in response to ovalbumin vaccination. This could be due to increased inflammation and immune activation caused by excess liver copper. Excess liver copper resulted in heightened severity of disease and an ablated nutritional immunity response compared to cattle with normal liver Cu concentrations. This suggests that copper over-supplementation is a potential contributor to the increased risk of dairy × beef calves to respiratory disease.

b. Introduction

Dairy × beef crossbred cattle have become an increasingly critical component of the U.S. beef industry, as beef originating from dairy herds accounts for 20.5-22.7% of US beef output (DelCurto et al., 2017). Bovine respiratory disease (**BRD**) is one of the most common and costly diseases in the beef industry, annually costing an estimated $3 billion on prevention and treatment (Griffin, 1997). Bovine respiratory disease poses an even greater threat in the dairy × beef sector. For example, a study of 800 cattle in a Kansas feedlot found dairy × beef crossbred cattle were twice as likely to succumb to BRD than native beef cattle (Theurer et al., 2020). This makes the integration of dairy × beef calves into feedlots a major challenge.

Dairy × beef crossbred calves are exposed to much higher Cu concentrations than native beef calves. A study examining 39 dairy operations in California with an average herd size of 787 cows found the median Cu supplementation rate was 17.2 mg/kg of diet DM, nearly 8 mg/kg higher than NRC (2001) recommendations. Unsurprisingly, reports of Cu toxicosis in dairy cows have increased significantly in recent years (Kendall et al., 2015). Further, in a study examining necropsy reports of 601 calves up to one year of age with causes of death unrelated to Cu toxicity, dairy calves had liver Cu concentrations 135-200 mg Cu/kg DM greater than beef calves, assuming the DM content of liver is 30% (Puschner et al., 2004). It is probable that excess Cu fed to dairy cows is translating to excess Cu in calves born to these cows, since Cu is transported to the fetus via the placenta (McArdle and Erlich, 1991). Adequate Cu status based on liver parameters ranges from 125-600 mg Cu/kg DM in cattle (Kincaid, 1999); however, it has been noted that oxidative damage can occur in the liver at Cu concentrations as low as 400 mg Cu/kg DM (Strickland et al., 2019).

Although copper (**Cu**) plays an integral role in enzyme function and antioxidant capacity, excess Cu is known to contribute to oxidative stress. As the liver's capacity to safely store Cu is exceeded, free Cu^{+} ions are released, which contribute to the generation of reactive oxygen species (Bremner, 1998). This could result in impaired immune system function. Pocino et al. (1991) showed that feeding high concentrations of Cu to mice impaired cellular and humoral immune responses, and pro-inflammatory cytokine production in response to sheep red blood cells decreased as Cu intake increased. Elevated Cu status impairs the response to influenza vaccination in young men and reduces the percentage of circulating neutrophils and serum IL-2R (Turnlund et al., 2004).

Based on the few previous studies conducted in other species and the greater Cu exposure dairy × beef calves face, it is possible that excess Cu status is contributing to immune dysfunction, but this hasn't yet been examined in dairy × beef calves. Thus, the objective of this

study was to determine the impact of liver Cu concentration on antibody production in response to a modified live respiratory vaccine (Bovilis Vista 5, Merck Animal Health, Madison, NJ) and the response to a bovine respiratory disease challenge.

c. Methods

All experimental procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (log no. 22-213).

Pre-trial Period

Sixty-four dairy × beef crossbred steers (209 ± 15.4 lb, approximately 8 weeks of age) were purchased from a single grower, but multiple source dairies, and delivered to the Iowa State University Beef Nutrition Farm (Ames, IA). Upon arrival, steers were dewormed (Safe-Guard oral suspension, Merck Animal Health, Madison, NJ) and blocked by body weight (**BW)** into eight pens (eight steers per pen). One pen from each weight block was assigned to one of two target liver Cu statuses: adequate Cu (**ADE**) or high Cu (**HIGH**). To achieve target Cu status, all steers were fed a pelleted diet (**Table 1**) containing 20 or 10 mg Cu/kg diet diem for ADE and HIGH, respectively, plus ad libitum hay. This pelleted diet was fed for 47 d before steers were gradually transitioned to a total mixed ration (**TMR, Table 2**), which was supplemented with 0 or 10 mg Cu from Cu sulfate/kg diet DM for ADE and HIGH, respectively. Due to health concerns, all steers were treated with Draxxin (Zoetis, Parsippany, NJ) 25 d after arrival. Steers were administered a zeranol implant (Ralgro, Merck Animal Health) 39 d after arrival. Liver biopsies were collected from all animals 81 d post-arrival to confirm Cu status aligned with treatment assignments, and steers were assigned to **Experiment 1** (*n* = 13 ADE and 15 HIGH) or **Experiment 2** (*n* = 13 ADE and 13 HIGH); 10 steers were not assigned to either experiment and removed from the study due to health concerns.

Experiment 1: Vaccine Challenge

Animals and Experimental Design

Day 0 of Experiment 1 began 89 days after steers initially arrived. Liver Cu for ADE averaged 291 ± 24 mg/kg DM (range 240-376 mg/kg DM), and HIGH averaged 665 ± 23 mg Cu/kg liver DM (range 519-893 mg/kg DM). Steers were weighed and administered a respiratory vaccine (Bovilis Vista 5, Merck Animal Health) as well as 2 mL subcutaneously of an ovalbumin vaccine (EndoFit™ OVA, InvivoGen, San Diego, CA) containing 2 mg of ovalbumin glycoprotein adjuvanted with a 15% v/v solution of Montanide Gel PR01 (Seppic, Courbevoie, France). Steers were weighed and boostered with both vaccinations 21 d later.

Sample Collection and Analytical Procedures

Weekly TMR samples were collected and dried in a forced air oven at 70°C to determine DM content, after which they were ground through a 2 mm screen (Retsch Zm100 grinder; Retsch GmBH, Haan, Germany). Samples were composited by month for further analysis. Liver biopsies were collected on d -7 as previously described by Engle and Spears (2000) and were transported on ice to the laboratory, after which they were stored at -20°C until further analysis. Blood was collected on d 0, 7, and 49 via jugular venipuncture into one serum separator tube and one K_2EDTA plasma tube (Becton Dickenson, Rutherford, NJ). Blood was transported to the lab and centrifuged at 1,000 × *g* for 20 min at 4°C, and serum and plasma were harvested and stored at -80°C and -20°C, respectively, until further analysis.

Plasma, liver, and monthly TMR composites were prepared and analyzed for Cu concentration via inductively coupled plasma-optical emissions spectrometry (**ICP-OES**; ICP Optima 7000 DV, Perkin Elmer, Waltam, MA) as described by Pogge and Hansen (2013).

Serum was analyzed by the Iowa State University Veterinary Diagnostic Laboratory for bovine respiratory syncytial virus (**BRSV**), bovine herpesvirus-1 (**BHV1**), bovine viral diarrheal virsus-1 (**BVDV1**), and bovine viral diarrheal virsus-2 (**BVDV2**) titers. Serum was also analyzed for haptoglobin via commercial enzyme-linked immunosorbent assay (**ELISA**; Immunology Consultants Laboratory, Inc, Portland, OR).

Ovalbumin (**OVA**) served as a model antigen to evaluate the immune response independent of factors such as maternal antibodies. Response to ovalbumin vaccination was analyzed via indirect ELISA using methods adapted from Rivera et al. (2002) and Chang et al (1996). Absorbances were read at 450 nm and 605 nm; 605 nm absorbances were subtracted from 450 nm absorbances to correct for background. The ratio of each corrected absorbance to the positive control was calculated and used for statistical analysis.

Statistical Analysis

Data were analyzed as a completely randomized design in the MIXED procedure of SAS 9.4 (SAS Inst. Cary, NC). Because liver Cu concentration was determined from each animal, steer was the experimental unit. All titer, OVA response, and haptoglobin data were transformed using the natural logarithm to meet assumptions for normality and analyzed with the fixed effect of Cu treatment and day as repeated. Interactions of Cu treatment, day, and treatment × day were analyzed. Body weight and ADG were analyzed with the fixed effect of Cu treatment. Cook's D statistic was used to test for outliers with a cutoff value of 0.5. Six single-timepoint samples were removed from haptoglobin analysis due to being high outliers (3 HIGH, 3 LOW). One steer (LOW) was removed from the trial due to unrelated health complications. Data shown are back-transformed LSMEANS and SEM.

Experiment 2: Bovine Respiratory Disease Challenge

Animals and Experimental Design

Day 0 of Experiment 2 began 120 d after steers initially arrived at the Beef Nutrition Farm. Steers in Experiment 2 were fed and treated during this time as described above for Experiment 1 steers. Liver biopsies were collected on d -18 and consecutive-day BW on d -2 and -1. Day -18 liver Cu averaged 290 ± 96 and 633 ± 85 mg Cu/kg liver DM, respectively. Steers were trucked for 6 hours before delivery at the Animal Resource Station (**ARS**) in Ames, IA on d -1. Upon arrival at ARS, all steers were supplemented with 5 mg Cu from Cu sulfate/kg diet DM, regardless of treatment, and treatments were housed in separate pens. Steers were infected with $10^4 TCID_{50}$ BRSV on d 0 using methods previously described by Sacco et al. (2012). On d 5, steers were infected with $5x10^8$ CFU *Mannheimia haemolytica*, prepared and administered as previously described (Capik et al., 2015; Hong et al., 2024).

Sample Collection and Analytical Procedures

Blood was collected on d 0, 5, 7, 10, and 13 via jugular venipuncture into serum separator tubes and plasma K_2EDTA tubes. Tubes were transported to the laboratory and processed as in

Experiment 1. Serum samples were used to determine haptoglobin concentrations as in Experiment 1 as well as Ferric Reducing Antioxidant Power (**FRAP**) on d 0, 7, and 13. Serum samples from d 13 were sent to the Iowa State University Veterinary Diagnostic Laboratory to be assessed for markers of liver damage. Plasma samples from d 0, 5, 7, 10, and 13 were analyzed via ICP-OES to determine plasma Cu, zinc (**Zn**), and iron (**Fe**) concentrations. Liver biopsies were collected on d 13 and analyzed for Cu concentrations as described in Experiment 1.

To determine gut permeability, 180 mM chromium-EDTA (**Cr-EDTA**) was prepared as previously described (Wood et al., 2015), and 500 mL were administered orally to each steer prior to infection on d 0 as well as on d 7. Four hours after dosing with Cr-EDTA, blood samples were collected into K_2EDTA plasma tubes via jugular venipuncture. Plasma was harvested and Cr concentrations were measured via ICP-OES as described in Experiment 1 with slight modifications: plasma was diluted 1:3 in 5% trace mineral grade nitric acid, and a stock solution (TruQ Chromium, PerkinElmer) with a concentration of 1000 µg Cr/mL was used to create standards to compare unknown samples against.

Clinical signs of disease were evaluated daily from d 0 (pre-infection) to d 13 by a single trained observer according to the DART system (depression, appetite, respiratory changes, and temperature; Griffin et al., 2010). In addition, rectal temperature was measured on d 0, 5, 7, 10, and 13.

Thoracic ultrasonography (**TUS**) was performed to determine lung consolidation in response to infection on d 0, 5, 7, 10, and 13 using an IBEX® EVO® (E.I. Medical Imaging, Loveland, CO) as previously described (Hong et al., 2024). A scoring system was modified from Rademacher et al. (2013), where fewer than five pleural defects are a score of 0, and five or more pleural defects but no consolidation receives a score of 1. Lung consolidation is scored 2-4 depending on the maximum depth of consolidation: a score of 1 is less than or equal to 2 cm, a score of 2 is between less than or equal to 2 cm but less than 4 cm, and a score of 3 is greater than or equal to 4 cm of consolidation.

Nasopharyngeal swabs were collected on d 0, 5, 7, 10, and 13 to determine *M. haemolytica* bacterial load. The swabs were stored in sterile 5 ml microtubes and transported to the laboratory on ice. In brief, 2 ml 1x minimum essential medium (Gibco, Waltham, MA) was added to each tube and vortexed. Approximately 1.2 ml of the washed media was recovered and transferred to a sterile microcentrifuge tube and was used as the inoculum to get obtain counts following a previously published protocol with slight modifications (Slate et al., 2021). Briefly, 100 µl of the inoculum was diluted 1:100 and 1:1000 to ensure countable colonies. One hundred µL of each dilution were spread onto blood agar plates (Remel, Lenexa, KS) and incubated at 37°C for 24 hours. Colonies with typical *M. haemolytica* morphology were counted from one of the dilutions and the colony forming units per ml (**CFU/ml**) was calculated.

Bronchoalveolar lavage (**BAL**) samples were collected on d 0 and 7 as previously described (Guerra-Maupome et al., 2019). All BAL fluids were transported on ice to the laboratory, filtered through sterile gauze, and centrifuged at 2,000 × *g* for 10 min, after which samples were stored in 24% glycerol for bacterial preservation. To determine bacterial recovery, preserved BAL samples were thawed and centrifuged at 3,000 × *g* for 10 min. Pelleted bacteria

were then resuspended in 100 µL of sterile saline, plated on blood agar plates, and cultured for 2 d prior to counting *M. haemolytica* bacterial colonies. Bronchoalveolar lavage cell pellets from each sample were also stored in TRIzol reagent (Invitrogen) and total RNA isolated via RNeasy spin columns according to manufacturer instructions (QIAGEN, Hilden, Germany), and 500 ng of RNA were used to synthesize cDNA. Power SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA) was used to perform qPCR as previously described, utilizing a ThermoFisher Quant Studio 5 Real-Time PCR machine. Primer information for target genes relating to Cu metabolism is displayed in **Table 3**; RPS9 was utilized as a housekeeper gene.

To determine viral RNA shedding, nasal swabs collected on d 5 and 7 were stored in serum-free minimal essential medium (Gibco) at -80°C until RNA isolation was performed via QIAGEN RNeasy kit according to manufacturer instructions. The BRSV NS2 gene was quantified from isolated RNA via qPCR using TaqMan-RNA-to-CT 1-step kit (Applied Biosystems) as previously described (McGill et al., 2018).

Statistical Analysis

The MIXED procedure of SAS 9.4 was used to analyze haptoglobin, FRAP, plasma trace mineral, nasopharyngeal swab bacterial load, and rectal temperature with the fixed effect of Cu treatment and day post infection as repeated measure. Bronchoalveolar lavage bacterial recovery, gene expression, markers of liver damage, and plasma Cr concentration were also analyzed in the MIXED procedure of SAS with the fixed effect of Cu treatment within day (d 0 or d 7). Because not all samples analyzed for Cr were collected precisely 4 hours after dosing, the exact time between dose and sample collection was used as a covariate in the analysis of plasma Cr concentrations. Haptoglobin, nasopharyngeal bacterial load, and BAL bacterial load were transformed using the natural logarithm to meet assumptions for normality; LSMEANS and SEM are back-transformed for discussion. Clinical scores were analyzed in the GLIMMIX procedure of SAS as categorical values with the fixed effect of Cu treatment and day post infection as repeated measure. At various timepoints throughout the disease challenge, 3 steers (1 ADE, 2 HIGH) required treatment and were removed from the study after being treated; data collected from these animals prior to treatment was included in statistical analysis. Cook's D statistic was used to test for outliers with a cutoff value of 0.5; however, none were detected.

d. Results

Experiment 1

Copper Concentrations

Prior to vaccination, liver Cu averaged 291 ± 24 and 665 ± 23 mg Cu/kg liver DM for ADE and HIGH, respectively. Plasma Cu concentrations on d 0, 21, and 49 are shown in **Figure 2** and were affected by day ($P < 0.01$), where plasma Cu did not differ between d 0 and 21 ($P = 0.17$) and was lesser on d 49 ($P < 0.01$). Plasma Cu concentration was not affected by treatment or treatment × day ($P \geq 0.39$).

Serum Antibody Titers

Serum antibody titers for BRSV, BHV1, BVDV1, and BVDV2 are shown in **Figure 2**. None of the titers measured were affected by treatment or treatment × day ($P \geq 0.12$). Bovine respiratory syncytial virus and BHV1 increased with sampling day ($P < 0.01$). Bovine viral diarrhea virus 2 was affected by day ($P < 0.01$) where titers were similar on d 0 and 21 ($P =$

0.14) and had increased by day 49 ($P < 0.01$). Titers for BVDV1 were not affected by treatment, day, or treatment × day ($P \geq 0.38$). Given the presence of titers on d 0 and the lack of response, it appears these steers were previously exposed to BVDV1.

Ovalbumin Response

The relative-to-positive ratios for ovalbumin vaccine response are presented in **Figure 3**. There was a treatment × day interaction ($P = 0.04$) where both treatments were similar on d 0 ($P = 0.16$), and on d 21, HIGH increased ($P < 0.01$), and ADE remained similar to d 0 ($P = 0.22$). On d 49, both treatments were similar ($P = 0.52$) and had increased compared to d 0 and 21 ($P \leq 0.04$).

Serum Haptoglobin Concentrations

Serum haptoglobin concentrations are presented in **Figure 2** and were not affected by treatment or treatment × day ($P \geq 0.47$) but were affected by day ($P < 0.01$). Haptoglobin concentrations were similar on d 0 and 21 ($P = 0.26$) and were decreased on d 49 ($P < 0.01$). Notably, there was a great deal of variation present within this dataset, especially on d 21, and this may have impacted the ability to detect treatment effects.

Experiment 2

Clinical Outcomes

Liver Cu prior to disease challenge averaged 279 ± 26 and 603 ± 28 mg Cu/kg liver DM for ADE and HIGH, respectively. Over the course of the disease challenge, three steers were removed from the study and treated due to health concerns. Reasons for removal included high rectal temperature, difficulty breathing, and lethargy. One HIGH steer was removed on d 3 post-infection and treated and was eventually euthanized. Another HIGH steer was removed and treated on d 5 and was not inoculated with *M. haemolytica.* One LOW steer was removed after sampling on d 7. Any steer that was removed from the study was included in statistical analysis up until the point of removal.

Bacterial Recovery

Bacterial recovery from BAL and nasopharyngeal swabs is presented in **Table 4**. On d 0, *M. haemolytica* was only recovered from the nasopharyngeal swab from one animal (HIGH); therefore, statistical analysis of bacterial recovery from nasopharyngeal swabs was only conducted on d 5, 7, 10, and 13. Bacterial recovery from nasopharyngeal swabs was not affected by treatment ($P = 0.95$) or treatment × day ($P = 0.23$) but was affected by day ($P < 0.01$), where recovery was highest on d 5 ($P < 0.01$), with the all other days post infection being similar ($P \geq 0.14$). *Manheimia haemolytica* recovery from BAL samples on d 0 and d 7 were not affected by treatment ($P \geq 0.14$)

Signs of Clinical Disease

Clinical scores are presented in **Figure 4** and were affected by treatment × day ($P = 0.04$), where both treatments are similar on d 0-3 ($P \leq 0.13$), but HIGH steers have greater clinical scores starting on d 4 that peak on d 6 ($P < 0.01$) and remain greater than ADE steers through d 14 ($P \leq 0.08$). Adequate steers increased in clinical scores gradually from d 4-7, with 7 being greater than 4 ($P > 0.01$) and 5 and 6 being intermediate. From d 8-14, ADE steers gradually return to pre-infection scores with 8 being greater than 14 ($P = 0.02$) and 9-13 being

intermediate. Clinical scores were also affected by treatment ($P < 0.01$) with HIGH having greater clinical scores than ADE.

Rectal temperatures are presented in **Figure 4** and were not affected by treatment or treatment × day ($P \leq 0.27$). Rectal temperatures were affected by day ($P = 0.02$), where they are highest on d 7 ($P \leq 0.04$) of the disease challenge and return to pre-infection temperatures on d 13, with d 10 being intermediate.

Lung consolidation scores obtained from TUS are displayed in **Figure 4** and were affected by day ($P < 0.01$), where d 0 and 5 were similar ($P = 0.25$) and less than d 7, 10, and 13 post-infection ($P < 0.01$). HIGH steers tended to have greater TUS scores than ADE overall ($P = 0.08$).

Trace Mineral Concentrations

Liver trace mineral concentrations prior to and after disease challenge are displayed in **Table 5**. By design, liver Cu concentrations differed by treatment on both timepoints, where HIGH had greater liver Cu concentrations then ADE ($P < 0.01$).

Plasma trace mineral status over the course of disease challenge is shown in **Figure 5**. Plasma Cu tended to have a treatment × day interaction ($P = 0.07$), where both treatments were similar ($P \geq 0.34$) and remained consistent from d 0 to d 5 ($P \geq 0.25$). LOW increased in plasma Cu from d 5 to d 7 ($P < 0.01$) remained consistent on d 10 ($P = 0.43$) and decreased on d 13 ($P = 0.04$). HIGH increased from d 5 to d 10 ($P = 0.03$), with d 7 being intermediate, and remained steady on d 13 (P 0.31). Plasma Zn affected by treatment × day ($P < 0.01$) where ADE had greater plasma Zn than HIGH on d 0 ($P = 0.01$). ADE remained steady through d 5 ($P = 0.18$) and decreased on d 7, 10, and 13 ($P \leq 0.03$). HIGH did not change from d 0 to d 10 ($P \geq 0.18$) and had increased plasma Zn from d 10 to d 13 ($P = 0.03$). Plasma Fe was not affected by treatment, day, or treatment × day ($P \geq 0.14$).

Gastrointestinal Tract Permeability

Plasma Cr concentrations measured on d 0 and 7 post infection are displayed in **Figure 7**. Greater plasma Cr concentration indicates increased paracellular movement across the intestinal epithelium; therefore, greater plasma Cr concentrations suggest greater gastrointestinal tract permeability (leaky gut). Chromium concentrations did not differ between treatment on either timepoint ($P \geq 0.22$).

Markers of Antioxidant Status

Ferric reducing antioxidant power and haptoglobin concentrations throughout disease challenge are displayed in **Figure 6**. Ferric reducing antioxidant power was not affected by treatment or treatment × day ($P \geq 0.19$). Day was significant ($P < 0.01$) where d 0, 7, and 13 were all different from each other ($P \geq 0.02$), with d 0 being the lowest, d 7 the greatest, and d 13 in between. Haptoglobin was not affected by treatment or treatment × day ($P \geq 0.52$) but was affected by day ($P < 0.01$) where d 0 and 13 were similar ($P = 0.99$) with the peak on d 7 ($P \leq 0.01$). Across all timepoints of this study, haptoglobin concentrations were markedly higher than what is typically seen in native beef animals.

Markers of Liver Damage

Markers of liver damage from blood samples collected on d 0 are displayed in **Table 6**. Excess liver Cu prior to disease challenge had no effect on aspartate aminotransferase, gamma-glutamyl transferase, creatinine, creatine kinase, total protein, or blood urea nitrogen ($P \geq 0.42$). Steers with adequate liver Cu had greater concentrations of total bilirubin, albumin, and glucose ($P \leq 0.03$) and tended to have greater concentrations of alkaline phosphatase ($P = 0.07$) than HIGH steers.

Gene Expression from Bronchoalveolar Lavage Cells

Gene expression data from cells recovered from BAL are displayed in **Table 7**. On d 0, there were no differences in expression between treatment for any gene ($P \geq 0.36$). On d 7, HIGH steers tended to have greater NRF2 (an oxidative stress gene) expression than ADE steers ($P = 0.06$). Expression of all other genes was similar between treatments ($P \geq 0.18$).

e. Discussion

Experiment 1

Dairy × beef cattle are frequently exposed to greater amounts of Cu than native beef cattle due to management practices in the dairy industry. Research across various species has indicates excess Cu contributes to liver damage and inflammation (Smedley et al., 2009; Humann-Ziehank et al., 2001), potentially altering immune function. The upper threshold for "adequate" liver Cu concentrations in cattle is 600 mg Cu/kg liver DM (Kincaid, 1999). In the present study, liver Cu concentrations in the HIGH treatment ranged from 519 to 894 mg Cu/kg liver DM.

Excess Cu did not result in increased haptoglobin, an inflammatory marker, in the HIGH treatment. However, haptoglobin levels decreased steadily over time in both treatments, which could indicate a pro-inflammatory event prior to d 0 that was resolving during the vaccination period, though the specific cause is unknown.

Previous work in humans has shown that excess Cu reduces the immune response to influenza vaccination (Turnlund et al., 2004). In contrast, both treatments in the present study responded equally well to vaccination against BRSV, BHV1, and BVDV2. The high titers for BVDV1 prior to vaccination indicate that these cattle were likely previously exposed to BVDV1 and did not have increased antibody production in response to vaccination. Since these calves may have had previous viral exposure or may have possessed maternal antibodies, an ovalbumin vaccine was administered to evaluate antibody production to a completely foreign antigen. Interestingly, calves in the HIGH treatment appeared to have a stronger initial antibody response to ovalbumin than those in the ADE group, though by d 49, titers were similar between groups. Despite no observed differences in haptoglobin concentrations between ADE and HIGH, it is possible that calves in the HIGH treatment still experienced liver inflammation.

If liver inflammation was present, it may have resulted in a greater release of pro-inflammatory cytokines, such as IL-6, which could have primed the adaptive immune system for a faster response to the ovalbumin vaccine. IL-6 is known to promote B-cell differentiation and recruitment, leading to increased antibody production (Dienz et al., 2009). This could explain the

greater antibody titers observed in HIGH on d 21. Human patients with Wilson's disease, a genetic disorder where Cu accumulates in the liver, were noted to have increased IgG, IgM, and antibodies against a model antigen (Członkowska and Milewski, 1976). Further, the authors found that leukocytes of Wilson's Disease patients have impaired bactericidal ability, and free Cu ions may inhibit cell-mediated immunity. The authors hypothesize Cu may have had an inhibitory effect on T cells, disturbing their interaction with B cells, which may lead to the overactivation of B cells. It is possible that a similar effect was occurring in the present study where B cells were overactivated in HIGH steers resulting in increased antibody production; however, further research is needed to better understand the role of excess liver Cu in modulating pro-inflammatory cytokine production and its impact on vaccine responses.

Experiment 2

For Experiment 2, a different set of dairy × beef steers from the same cohort as Experiment 1 were used. These calves averaged 279 and 603 mg Cu/kg liver DM for ADE and HIGH, respectively. HIGH steers showed more signs of clinical disease from d 4 post-infection through the end of the trial, as evidenced by their elevated clinical scores. HIGH also had the greatest clinical scores on d 6 post-infection, which aligns with the expected peak in severity of disease symptoms. Calves in ADE experienced much less severe increases in clinical scores and returned to pre-infection scores by the end of the 13 d study, while HIGH had not. Further, HIGH tended to have greater TUS scores, indicating increased damage and consolidation in the lungs in response to disease.

Plasma Cu and Zn responded differently to disease between treatments. HIGH steers do not change plasma Cu concentrations throughout the course of disease, while ADE steers experience a slight increase in plasma Cu on d 10. Plasma Zn also does not change in HIGH steers, while ADE steers decrease in plasma Zn, especially on d 7 and 10 post-infection, during the expected peak of clinical disease. The changes in plasma Cu and Zn in the ADE treatment are consistent with a nutritional immunity response that has been well demonstrated in previous studies. In cattle, both Galarza et al. (2021) and Hong et al. (2024) noted decreases in plasma Zn concentrations following *M. haemolytica* infection, and Orr et al. (1990) found decreased plasma Zn following bovine rhinotracheitis infection. This decrease in plasma Zn in response to infection and inflammation is thought to starve pathogens of Zn, slowing the progression of disease. More recently, it has been suggested that this decrease in plasma Zn concentrations during infection is part of a cell signaling process in which Zn is recruited to tissues to serve as a signal to enhance immune cell activation (Kim and Lee, 2021). Meanwhile, plasma Cu increases with the nutritional immunity response, as Cu-containing ceruloplasmin is released as part of the acute phase response (Orr et al., 1990). Given the lack of nutritional immunity response in the HIGH treatment and their elevated clinical scores, excess Cu appears to hinder the response to infection, resulting in more severe signs of clinical disease.

Despite their elevated clinical scores and lack of nutritional immunity response, the HIGH treatment did not have greater haptoglobin concentrations than ADE. Similar to Experiment 1, haptoglobin concentrations were widely variable across both treatments. Disease challenge resulted in haptoglobin concentrations on d 7 that were over 10-fold greater than the steers from Experiment 1 experienced at any point throughout the vaccine period, suggesting disease challenge caused severe inflammation. Hong et al. (2024) infected native beef steers

weighing approximately 694 lb with *M. haemolytica* and noted haptoglobin concentrations peaked at approximately 18 µg/mL 2 d post infection. Though not a perfect comparison because the steers in Experiment 2 used a BRSV and *M. haemolytica* co-infection model and were 200 lb lighter, the steers in the present study peaked at over 500 µg/mL 2 d following *M. haemolytica* infection. This may suggest dairy × beef animals exhibit more severe inflammation in response to disease than native beef steers, which would explain why dairy × beef animals are more likely to succumb to respiratory disease.

Ferric reducing antioxidant potential was measured in Experiment 2 to determine total circulating antioxidant capacity. Both treatments have decreased antioxidant capacity on d 7, which is concurrent with the increased inflammation at the same timepoint. Contrary to our hypothesis, excess liver Cu did not lead to decreased FRAP; however, while not statistically significant, it is interesting to note that the decrease in FRAP from d 0 to d 7 is more pronounced in HIGH than in ADE (28% decline in HIGH vs 14% in ADE). This may suggest excess Cu influences antioxidant status, but more research is needed to confirm this. In our prior transit stress work, FRAP declines and markers of inflammation increase in response to trucking, supporting a relationship between FRAP and inflammation. If excess Cu does decrease FRAP, it may follow that excess Cu increases inflammation (Deters and Hansen, 2020). Research on antioxidant capacity and inflammation in similar animals and challenge models to the present study is lacking, and more research is needed to confirm the different inflammatory response in dairy × beef and native beef calves and determine why dairy influenced animals seem to be more pro-inflammatory.

Steers with excess liver Cu have a different blood metabolite profile than those with normal Cu concentrations. HIGH steers had lesser serum albumin concentrations than ADE steers. Both treatments were within previously reported reference ranges for healthy adult cattle (Knowles et al., 2000). Wilson's disease has been shown to lead to decreased serum albumin as a function of liver damage induced by excess liver Cu (Boga et al., 2017). While HIGH Cu cattle in the present study are still within the normal range, it is possible they had decreased albumin due to liver damage caused by excess Cu. Both treatments had total bilirubin concentrations well in excess of previously reported average concentrations (Doornenbal et al., 1988; Mohri et al., 2007). Bilirubin is formed from the breakdown of hemoglobin in red blood cells and excreted in the bile(Wang et al., 2006), and elevation can be an indicator of liver damage, bile duct obstruction, or elevated red blood cell turnover. ADE steers had greater total bilirubin concentrations than HIGH, but the mechanisms driving this are unknown.

Alkaline phosphatase concentrations for both treatments are on the upper threshold of the healthy reference range (Aiello and Moses, 2016); however, Knowles et al. (2000) have shown that serum ALP concentrations are highest at birth and decrease steadily with age. The tendency for ADE calves to have increased ALP may be a function of Zn status, as ALP is a Zn-dependent enzyme (Yamaguchi and Yamaguchi, 1986). The fact that the ADE treatment had greater circulating Zn than HIGH may have also meant ADE had more Zn available to support ALP in circulation.

Intriguingly, HIGH steers had markedly lower concentrations of circulating glucose, which could be attributed to the increased glucose requirements of an activated immune system.

Kvidera et al. (2017) administered lipopolysaccharide (**LPS**), a potent pro-inflammatory agent, to lactating Holstein cows and determined that acute inflammation caused hypoglycemia. The authors further quantified the glucose requirement of LPS-challenged cattle and found these animals required over 1 kg of glucose to be infused over 720 minutes to maintain serum glucose concentrations equivalent to non-LPS administered controls (Kvidera et al., 2017). If the HIGH steers had greater inflammation than ADE, the immune system may be utilizing glucose at a greater rate than it can be synthesized in the liver, leading to decreased glucose in circulation compared to ADE. Also, circulating glucose in ruminants is largely driven by hepatic gluconeogenesis (Glucose Metabolism in Ruminants, 1976), so differences in serum glucose may be a result of differences in liver function. It is possible that excess liver Cu decreased hepatic glucose production, contributing the decreased serum glucose concentrations in HIGH, but this cannot be attributed directly to liver damage based on the data collected.

Steers with excess liver Cu had greater expression of NRF2 in cells recovered from BAL on d 7, indicating a cellular response to oxidative stress. Taken into consideration with the increased clinical scores and ablated nutritional immunity response in the HIGH treatment, this could indicate that excess Cu heightened the severity of respiratory disease, leading to more oxidative stress in the respiratory tract which the steers were responding to by increasing antioxidant production via NRF2 transcription. Further, the numerically greater decline in FRAP experienced by the HIGH treatment also could indicate an increased need for antioxidant production. Perhaps, since signs of clinical disease were more moderate in the ADE treatment, less antioxidant production was needed and less NRF2 transcription was detected. Chromium-EDTA was used to determine the effect of excess Cu on total gastrointestinal tract permeability on d 0 and 7 of disease challenge. No treatment differences in plasma Cr concentrations 4 h after dosage were noted on either day; however, HIGH had numerically greater Cr concentrations on d 0 than ADE. This may suggest that dairy × beef cattle with very high liver Cu concentrations may not respond well to transit stress, with implications for post-arrival feed intake and health management, but more research with greater statistical power is needed to confirm this.

In conclusion, excess liver Cu resulted in an ablated nutritional immunity response to infection and heightened severity of disease. Although haptoglobin did not reveal differences in inflammation between treatments, it appears excess liver Cu concentrations may contribute to the poor health of dairy × beef calves in feedlots. This work indicates that over-fortifying diets with Cu may has negative impacts on animal health, especially in dairy × beef calves which have greater exposure to Cu and heightened susceptibility to disease.

f. Tables and Figures

Table 1. Analyzed composition of pelleted diet fed for 47 d after arriving at the Iowa State University Beef Nutrition Farm.

Analyzed Composition[1]	High Cu pellet	Low Cu pellet
Crude protein, % DM[2]	25.3	25.1
Neutral detergent fiber, % DM[2]	16.9	16.5
Ether extract, % DM[2]	3.8	4.0
Sulfur, % DM[3]	0.45	0.42
Molybdenum, mg/kg DM[3]	1.08	1.59
Copper, mg/kg DM[4]	22.8	8.8
Zinc, mg/kg DM[4]	54.0	56.5
Iron, mg/kg DM[4]	184	183

[1]Weekly samples were collected, dried in a forced air oven at 70°C, ground, and composited by month for analysis.
[2]Analysis performed by Dairyland Laboratories (Arcadia, WI)
[3]Analysis performed via inductively coupled plasma-mass spectrometry by the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA).
[4]Analysis performed via inductively coupled plasma-optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA).

Table 2. Diet composition of total mixed ration.

Ingredient	% of diet DM
Hay	15
Corn	15
Corn Silage	15
Dried distillers grains	18.06
Sweet Bran[1]	35
Cu premix[2]	5
Trace mineral premix[3]	0.0204
Limestone	1.5
Salt	0.31
Vitamin A & E premix[4]	0.1
Rumensin 90	0.0135
Analyzed Composition	
Crude protein, %[5]	18.4
Neutral detergent fiber, %[5]	33.1
Ether extract, %[5]	5.2
Sulfur, %[6]	0.31
Molybdenum, mg/kg DM[6]	0.97
Copper, mg/kg DM[7]	4.8
Zinc, mg/kg DM[7]	67.5
Iron, mg/kg DM[7]	211

[1]Branded wet corn gluten feed (Cargill Milling, Blair, NE)
[2]Cu treatments were included as a dried distillers grains-based premix that replaced dried distillers grains in the diet. Treatments included: no supplemental Cu (ADE), 10 mg Cu/kg diet DM (HIGH), and 5 mg Cu/kg diet DM (fed to both treatments in Experiment 2 during disease challenge).
[3]Trace mineral premix was formulated to supplement all trace minerals other than Cu at NASEM (2016) recommendations.
[4]Vitamin A & E premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet DM.
[5]Analysis of ADE TMR composite by Dairyland Laboratories (Arcadia, WI).
[6]Analysis of ADE TMR composite by the Iowa State University Veterinary Diagnostic Laboratory (Ames, IA).
[7]Analyzed Fe, Zn, and Cu represents ADE dietary treatment with no supplemental Cu and were analyzed via inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). All treatments were supplemented Zn and Fe to meet NASEM (2016) recommendations. Copper treatments were supplemented in addition to Cu in the basal diet.

Table 3. Forward and reverse primers for quantitative real-time polymerase chain reaction in cells recovered from bronchoalveolar lavage samples.

Gene	Function	Primer Sequence (5' - 3')	Accession No.
CTR1[2]	Primary route of copper import	F: AAGAGTCCTGGAGGTGTG	NM001100381.1
		R: GGTCAGATGAAGTGGTTGG	
LOX[3]	Copper-dependent; extracellular matrix development and repair	F: AGCTATTTGGTGCCTGAGTC	NM173932
		R: ATATGCGTGATGTCCTGTGTAG	
MMP9[4]	Zinc dependent; collagen degradation	F: GGTATGGGTAATGAAATTGATGTG	XM592003.4
		R: TCAGCAGCGATGTAGTAGTG	
MT2A[6]	Copper storage and protection from oxidative stress	F: GACCCCAGCCTCCAGTTCAGCTC	NM001075140.1
		R: CTTTGCATTTGCAGGAGCCGGC	
NRF2[7]	Antioxidant	F: CCCAGTCTTCACTGCTCCTC	NM001011678
		R: TCAGCCAGCTTGTCATTTTG	
RPS9[8]	Housekeeping gene	F: CCTCGACCAAGAGCTGAAG	DT860044
		R: CCTCCAGACCTCACGTTTGTTC	

[1]Copper transporter 1.
[2]Lysyl oxidase.
[3]Matrix metallopeptidase-9.
[4]Metallothionein 2A.
[5]Nuclear factor erythroid-2 related factor 2.
[6]Ribosomal protein subunit 9.

Table 4 (Experiment 2). Effect of liver Cu on bovine respiratory syncytial virus RNA recovery and *M. haemolytica* recovery from nasopharyngeal swabs and bronchoalveolar lavage samples in dairy × beef crossbred steers.

	Treatment[1]				Day		
Item	ADE	HIGH	SEM[2]	Treatment *P*-value		SEM	Day *P*-value
Nasopharyngeal swab *M. Haemolytica* count, CFU/mL[3]				0.95			< 0.01
d 0	0	0	-		0	-	
d 5	19	2	35		7[b]	8	
d 7	929	3695	5976		1853[a]	2042	
d 10	22402	6042	34709		11634[a]	13066	
d 13	898	10306	16776		3041[a]	3426	
Bronchoalveolar lavage *M. Haemolytica* count, CFU/mL[4]							
d 0	119	12	127	0.14	37	29	-
d 7	3	8	14	0.69	5	6	

[1]Treatments included HIGH liver Cu and ADE liver Cu (603 and 279 mg Cu/kg liver DM prior to challenge, respectively).
[2]Highest SEM of either treatment.
[3]Nasopharyngeal swabs were collected on d 0, 5, 7, 10, and 13. Bacterial recovery was analyzed with the main effects of treatment, day, and their interaction. *P*-values for main effects of treatment and day are shown; treatment × day was not significant ($P = 0.23$). For the day effect, values with unlike superscripts differ by day ($P \leq 0.05$).
[4]Bronchoalveolar lavage samples were collected on d 0 and 7, and data was analyzed with the main effect of treatment within day.

Table 5 (Experiment 2). Liver Cu concentrations of ADE and HIGH treatments prior to and following disease challenge.

	Treatment[1]			
Item	ADE	HIGH	SEM[2]	Treatment *P* - value
Liver Cu, mg/kg DM[3]				
d -18	279	603	27.8	< 0.01
d 13	250	516	32.6	< 0.01

[1]Treatments included HIGH liver Cu and ADE liver Cu (603 and 279 mg Cu/kg liver DM prior to challenge, respectively).
[2]Highest SEM of either treatment.
[3]Liver Cu measured via inductively coupled plasma-optical emission spectrometry (ICP Optima 7000 DV; Perkin Elmer, Waltham, MA).

Table 6 (Experiment 2). Effect of excess liver Cu on markers of liver damage.

	Treatment[1]			
Item[2]	ADE	HIGH	SEM[3]	Treatment *P* - value
Aspartate aminotransferase, IU/L[4]	4.45	4.41	5.790	0.65
Gamma-glutamyl transferase, IU/L	35.1	35.8	1.48	0.74
Alkaline phosphatase, IU/L	183.9	148.7	13.26	0.07
Creatinine, mg/dL	0.84	0.88	0.026	0.42
Creatine kinase, IU/L[4]	160.0	143.0	18.77	0.50
Total bilirubin, mg/dL	0.55	0.45	0.022	<0.01
Albumin, g/dL	3.00	2.85	0.045	0.03
Total protein, g/dL	7.33	7.39	0.165	0.79
Glucose, mg/dL	68.7	49.1	3.32	<0.01
Blood urea nitrogen, mg/dL	8.69	8.15	0.402	0.35

[1]Treatments included HIGH liver Cu and ADE liver Cu (603 and 279 mg Cu/kg liver DM prior to challenge, respectively).
[2]Serum samples collected immediately prior to infection were sent to the Iowa State University Clinical Pathology Laboratory (Ames, IA), where markers of liver damage were measured.
[3]Highest SEM of either treatment.
[4]Estimates were transformed using the natural logarithm to meet assumptions for normality. Presented data are back-transformed LSMEANS and SEM.

Table 7 (Experiment 2). The effect of excess liver Cu on relative expression[1] of copper metabolism and anti-inflammatory genes in cells recovered from bronchoalveolar lavage samples on d 0 and d 7 of disease challenge.

	Treatment[2]			
Item	ADE	HIGH	SEM[3]	Treatment *P* - value
d 0				
CTR1[4]	1.153	0.981	0.1819	0.65
LOX[5]	1.154	1.453	0.3420	0.90
MMP9[6]	2.149	6.510	3.5257	0.36
MT2A[7]	1.056	1.262	0.8915	0.62
NRF2[8]	1.097	0.968	0.0803	0.66
d 7				
CTR1[4]	1.101	1.311	0.1368	0.18
LOX[5]	1.421	1.296	0.3670	0.83
MMP9[6]	2.479	5.413	2.7624	0.35
MT2A[7]	1.283	0.910	0.2404	0.58
NRF2[8]	1.109	1.328	0.0751	0.06

[1]Relative expression was determined using the 2^-ΔΔCT method, where CT values were normalized to the housekeeper gene (RPS9). Then, the average ΔCT value from the ADE treatment was subtracted from the ΔCT value of each target to obtain ΔΔCT. Statistical analysis was performed on ΔCT values.
[2]Treatments included HIGH liver Cu and ADE liver Cu (603 and 279 mg Cu/kg liver DM prior to challenge, respectively).
[3]Highest SEM of either treatment.
[4]Copper transporter 1.
[5]Lysyl oxidase.
[6]Matrix metallopeptidase-9.
[7]Metallothionein 2A.
[8]Nuclear factor erythroid-2 related factor 2.

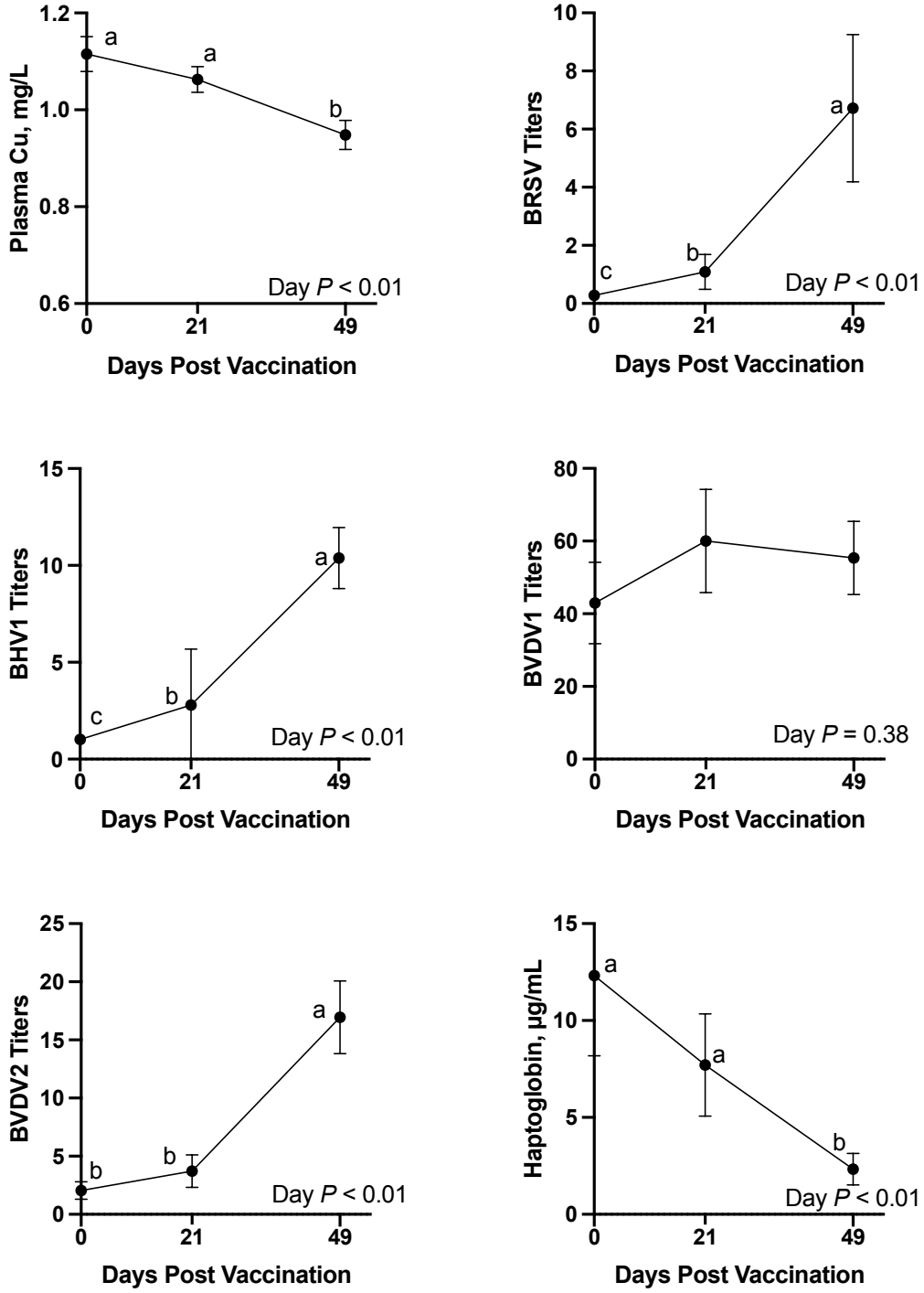

Figure 1 (Experiment 1). The effect of day relative to vaccination (Bovilis Vista 5 SQ, Merck Animal Health, Teaneck, NJ) on plasma Cu concentration, serum antibody titers, and serum haptoglobin concentration in dairy × beef crossbred steers. Within a card, data points with unlike

superscripts differ by day ($P \leq 0.05$). No day effect was noted for BVDV1 titers, and no significant treatment or treatment × day effects were noted for any viral titers, haptoglobin, or plasma Cu.

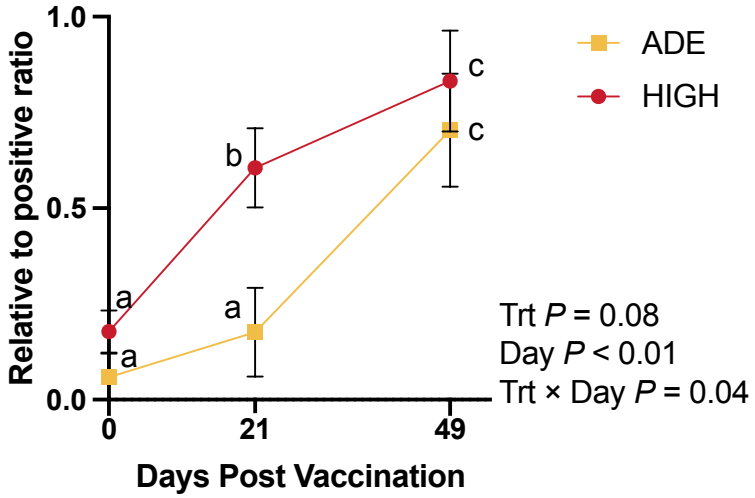

Figure 2 (Experiment 1). Effect of liver Cu × day ($P = 0.04$) relative to ovalbumin vaccination on ovalbumin antibody production in lightweight dairy × beef crossbred steers. Data points with unlike superscripts differ by treatment × day ($P \leq 0.05$).

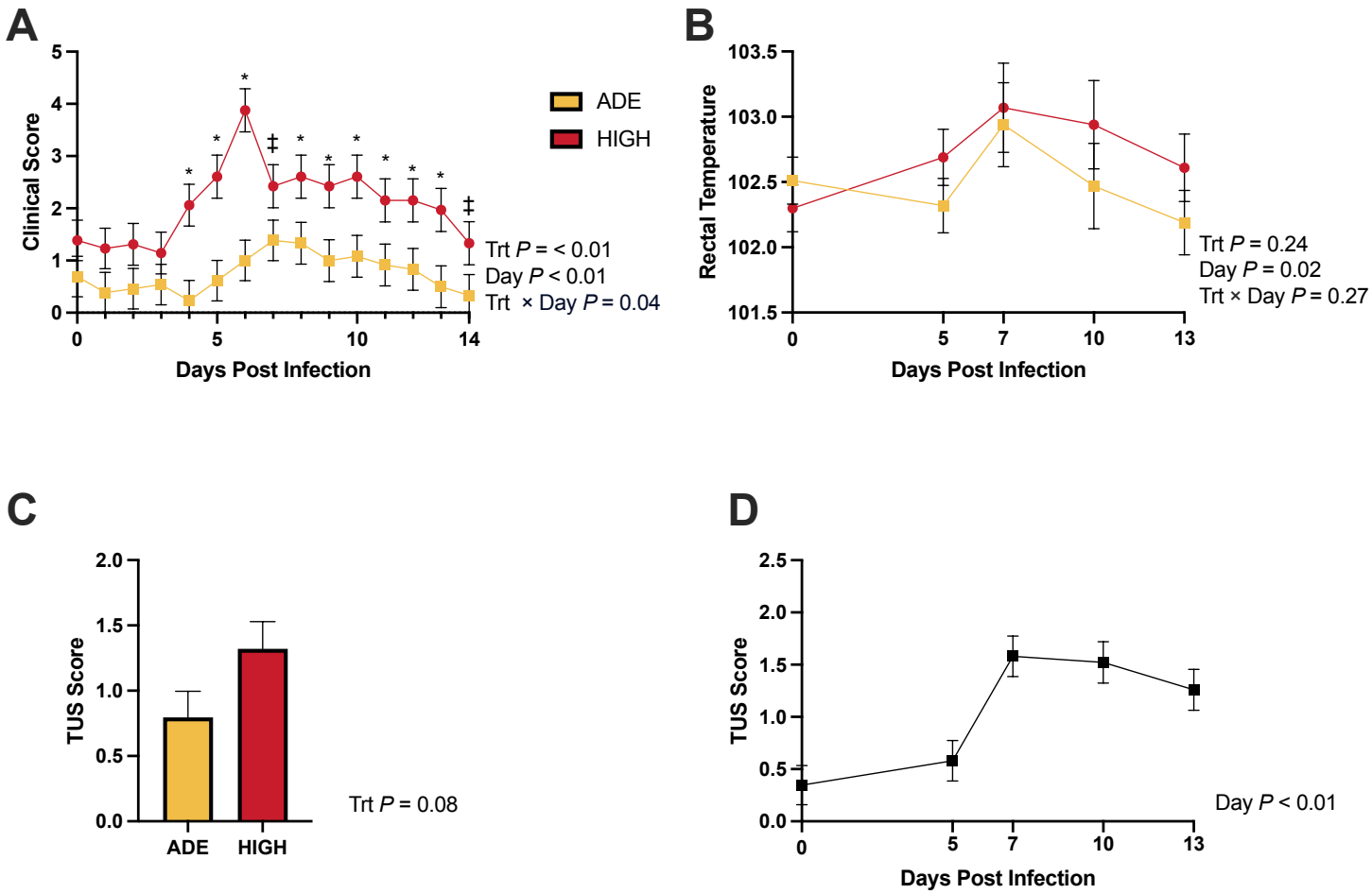

Figure 3 (Experiment 2). Effect of excess liver Cu on clinical disease scores (**A**), rectal temperatures (**B**), and thoracic ultrasonography scores (TUS; **C & D**) over the course of disease challenge in dairy × beef crossbred steers. **A** Clinical scores were recorded on d 0-14 post infection by a single trained observer according to the DART system. Within day post infection, * indicates significant difference between ADE and HIGH ($P \leq 0.05$) and ‡ indicates tendencies ($0.05 < P \leq 0.10$). **B** Rectal temperature was measured on d 0, 5, 7, 10, and 13 post infection. **C,D** Thoracic ultrasonography was conducted using an IBEX® EVO® (E.I. Medical Imaging; Loveland, CO) on d 0, 5, 7, 10, and 13 post infection with scores assigned based on the degree of lesions and consolidation in the lung. Scores tended to be affected by treatment (**C**) and were affected by day (**D**), but were not affected by treatment by day.

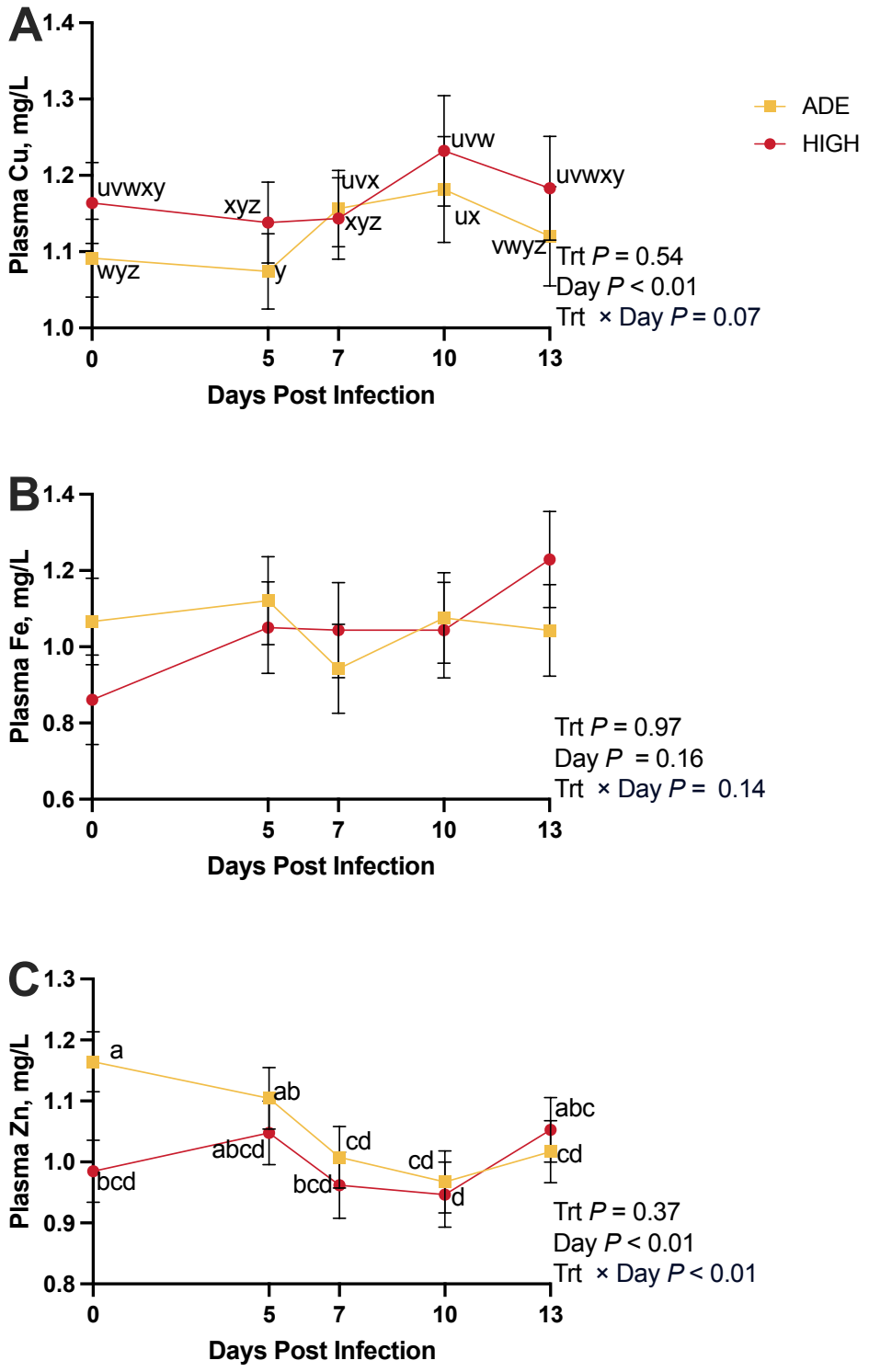

Figure 4 (Experiment 2). The effect of excess liver Cu, day relative to infection, and their interaction on plasma Cu (**A**), plasma Fe (**B**), and plasma Zn (**C**) in blood samples collected on d 0, 5, 7, and 10 post infection. Analysis was performed using inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV, Perkin Elmer, Waltham, MA). **A** Terms with unlike superscripts tend differ ($P \leq 0.1$). **C** Terms with unlike superscripts differ ($P \leq 0.05$).

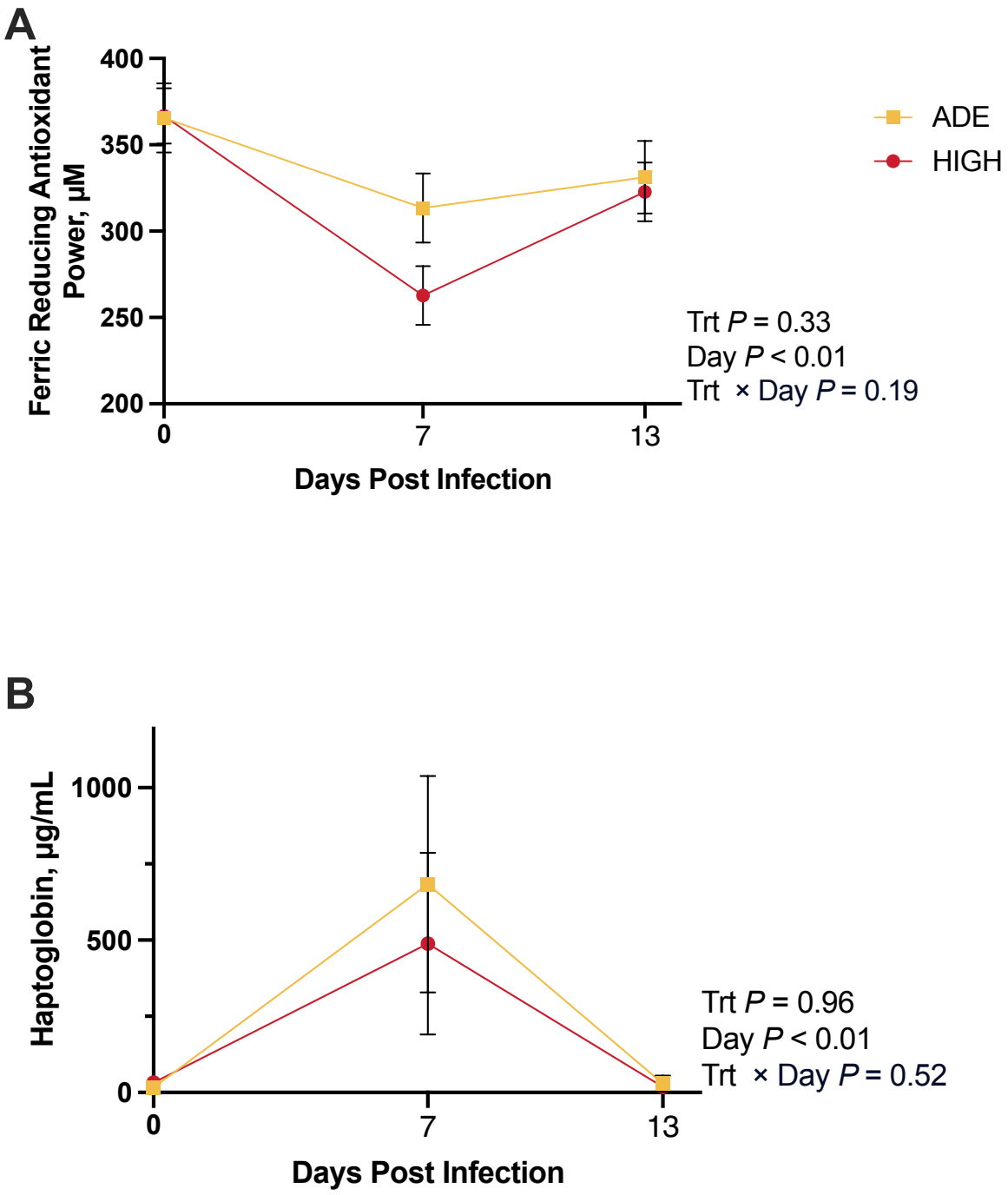

Figure 5 (Experiment 2). Effect of excess liver Cu on ferric reducing antioxidant power (FRAP; **A**) and haptoglobin concentration (**B**) over the course of disease challenge in dairy × beef crossbred steers. **A** Ferric reducing antioxidant power in serum samples collected on d 0, 7 and 13 post infection were measured via calorimetric assay (Arbor Assays; Ann Arbor, MI). **B** Haptoglobin concentrations of serum samples on d 0, 7, and 13 was determined using commercial enzyme linked immunosorbent assay (ICL Laboratories; Portland, OR).

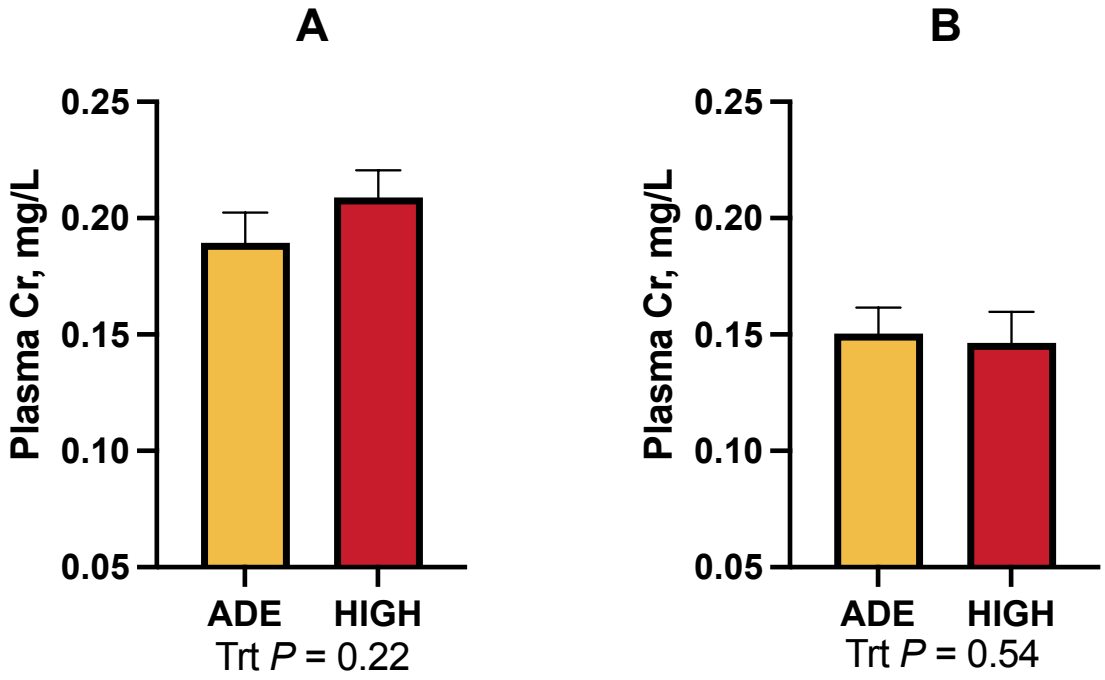

Figure 6 (Experiment 2). The effect of excess liver copper on gut permeability in dairy × beef crossbred steers 0 (**A**) and 10 (**B**) days after disease challenge. Chromium (Cr) EDTA was used as a marker for gut permeability. Five hundred mL of Cr EDTA were orally administered to steers prior to infection on d 0 and 7 d post infection. Four hours after dosing, blood samples were drawn for analysis of plasma Cr concentration via inductively coupled plasma optical emission spectrometry (ICP Optima 7000 DV; Perkin Elmer, Waltham, MA).

g. Unexpected Problem or Outcomes

None to report

h. Presentations (completed)

- Presentation of Experiment 1 at the American Society of Animal Science Midwest Section Annual Meeting. Madison, WI March

i. Publications (planned)

- Experiment 1 and Experiment 2 will be published as two separate manuscripts by the end of 2024.

j. Personnel Support

- Jodi McGill
  - Associate professor salary: $4579
- Stephanie Hansen
  - Professor salary: $3081
- Jacob Henderson
  - Graduate student salary: $29,558
  - Graduate tuition: $6864
- Luke Ellias – Research Technician salary: $18,066
- Undergraduate hourly labor: $5,513

k. Budget

Category	Budgeted	Spent
Personnel salaries and benefits	$45,197	$60,797
Publication costs	$2,000	$0
Travel	$2,000	$273
Animal housing costs	$18,956	$9,879
Veterinary diagnostic lab charges	$16,500	$2,619
Materials and supplies	$19,016	$23,238
Graduate Student Tuition	$6,864	$6,864
Total	$103,669	$103,669

l. Literature Cited

Aiello, S. E., and M. A. Moses. 2016. The Merck Veterinary Manual, 11th Edition. 11th ed. Wiley, Hoboken.

Boga, S., A. Ala, and M. Schilsky. 2017. Hepatic Features of Wilson Disease. In: Handbookof Clinical Neurobiology. Vol. 142. 3rd ed. Elsevier, Amsterdam. p. 21–97.

Bremner, I. 1998. Manifestations of copper excess. Am J Clin Nutr. 67:1069S-1073S. doi:10.1093/ajcn/67.5.1069S.

Capik, S. F., B. J. White, B. V. Lubbers, M. D. Apley, D. A. Mosier, R. L. Larson, and R. W. Murray. 2015. Characterization of *Mannheimia haemolytica* in beef calves via nasopharyngeal culture and pulsed-field gel electrophoresis. Journal of Veterinary Diagnostic Investigation. 27:568–575. doi:10.1177/1040638715597724.

Chang, G. X., B. A. Mallard, D. N. Mowat, and G. F. Gallo. 1996. Effect of Supplemental Chromium on Antibody Responses of Newly Arrived Feeder Calves to Vaccines and Ovalbumin. Can. J. Vet. Res. 60:140–144.

Członkowska, A., and B. Milewski. 1976. Immunological observations on patients with Wilson's disease. J Neurol Sci. 29:411–421. doi:10.1016/0022-510X(76)90188-X.

DelCurto, T., Murphy T., and Moreaux S. 2017. Demographics and long-term outlook for Western US beef, sheep, and horse industries and their importance for the forage industry. 47th Western Alfalfa & Forage Symposium. UC Cooperative Extension, Plant Sciences Department, University of California, Davis., Reno, NV.

Deters, E. L., and S. L. Hansen. 2020. Pre-transit vitamin C injection improves post-transit performance of beef steers. Animal. 14:2083–2090. doi:10.1017/S1751731120000968.

Dienz, O., S. M. Eaton, J. P. Bond, W. Neveu, D. Moquin, R. Noubade, E. M. Briso, C. Charland, W. J. Leonard, G. Ciliberto, C. Teuscher, L. Haynes, and M. Rincon. 2009. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. Journal of Experimental Medicine. 206:69–78. doi:10.1084/jem.20081571.

Doornenbal, H., A. K. W. Tong, and N. L. Murray. 1988. Reference Values of Blood Parameters in Beef Cattle of Different Ages and Stages of Lactation. Can. J. Vet. Res. 52:99–105.

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J Anim Sci. 78:2446. doi:10.2527/2000.7892446x.

Galarza, E. M., R. M. Lizarraga, N. Streitenberger, G. Arriaga, G. Abraham, G. A. Mattioli, J. M. Anchordoquy, and L. E. Fazzio. 2021. Assessment of Plasma Zinc and Total Leukocyte Count in Calves Experimentally Infected with Mannheimia haemolytica. Biol Trace Elem Res. 199:120–125. doi:10.1007/s12011-020-02145-4.

Glucose Metabolism in Ruminants. 1976. In: Third International Conference on Production Disease in Farm Animals. Wageningen. p. 25–29.

Griffin, D. 1997. Economic Impact Associated with Respiratory Disease in Beef Cattle. Veterinary Clinics of North America: Food Animal Practice. 13:367–377. doi:10.1016/S0749-0720(15)30302-9.

Griffin, D., M. M. Chengappa, J. Kuszak, and D. S. McVey. 2010. Bacterial Pathogens of the Bovine Respiratory Disease Complex. Veterinary Clinics of North America: Food Animal Practice. 26:381–394. doi:10.1016/j.cvfa.2010.04.004.

Guerra-Maupome, M., D. X. Vang, and J. L. McGill. 2019. Aerosol vaccination with Bacille Calmette-Guerin induces a trained innate immune phenotype in calves. PLoS One. 14:e0212751. doi:10.1371/journal.pone.0212751.

Hong, S., E. L. Rients, C. E. Franco, S. L. Hansen, and J. L. McGill. 2024. Impact of an Injectable Trace Mineral Supplement on the Immune Response and Outcome of Mannheimia haemolytica Infection in Feedlot Cattle. Biol Trace Elem Res. doi:10.1007/s12011-024-04251-z. Available from: https://doi.org/10.1007/s12011-024-04251-z

Humann-Ziehank, E., M. Coenen, M. Ganter, and K. Bickhardt. Long-Term Observation of Subclinical Chronic Copper Poisoning in Two Sheep Breeds. doi:10.1046/j.1439-0442.2001.00376.x. Available from: https://onlinelibrary.wiley.com/doi/10.1046/j.1439-0442.2001.00376.x

Kendall, N. R., H. R. Holmes-Pavord, P. A. Bone, E. L. Ander, and S. D. Young. 2015. Liver copper concentrations in cull cattle in the UK: are cattle being copper loaded? Veterinary Record. 177:493–493. doi:10.1136/vr.103078.

Kim, B., and W. W. Lee. 2021. Regulatory Role of Zinc in Immune Cell Signaling. Mol Cells. 44:335–341. doi:10.14348/MOLCELLS.2021.0061.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminants: A review. Proceedings of the American Society of Animal Science. 77:1–10.

Knowles, T. G., J. E. Edwards, K. J. Bazeley, S. N. Brown, A. Butterworth, and P. D. Warriss. 2000. Changes in the blood biochemical and haematological profile of neonatal calves with age. Veterinary Record. 147:593–598. doi:10.1136/vr.147.21.593.

Kvidera, S., E. Horst, M. Abuajamieh, E. Mayorga, M. Sanz Fernandez, and L. Baumgard. 2017. Glucose requirements of an activated immune system in lactating Holstein cows. doi:10.3168/jds.2016-12001. Available from: https://doi.org/10.3168/jds.2016-12001

McArdle, H. J., and R. Erlich. 1991. Copper Uptake and Transfer to the Mouse Fetus during Pregnancy. J Nutr. 121:208–214. doi:10.1093/jn/121.2.208.

McGill, J. L., S. M. Kelly, P. Kumar, S. Speckhart, S. L. Haughney, J. Henningson, B. Narasimhan, and R. E. Sacco. 2018. Efficacy of mucosal polyanhydride nanovaccine against respiratory syncytial virus infection in the neonatal calf. Sci Rep. 8. doi:10.1038/s41598-018-21292-2. Available from: www.nature.com/scientificreports/

Mohri, M., K. Sharifi, and S. Eidi. 2007. Hematology and serum biochemistry of Holstein dairy calves: Age related changes and comparison with blood composition in adults. Res Vet Sci. 83:30–39. doi:10.1016/J.RVSC.2006.10.017.

National Research Council (U.S.). Subcommittee on Dairy Cattle Nutrition. Nutrient requirements of dairy cattle.

Orr, C. L., D. P. Hutcheson, R. B. Grainger, J. M. Cummins, and R. E. Mock. 1990. Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. J Anim Sci. 68:2893. doi:10.2527/1990.6892893x.

Pocino, M., L. Baute, and I. Malavé. 1991. Influence of the oral administration of excess copper on the immune response. Fundamental and Applied Toxicology. 16:249–256. doi:10.1016/0272-0590(91)90109-H.

Pogge, D. J., and S. L. Hansen. 2013. Supplemental vitamin C improves marbling in feedlot cattle consuming high sulfur diets. J Anim Sci. 91:4303–4314. doi:10.2527/jas.2012-5638.

Puschner, B., M. C. Thurmond, and Y.-K. Choi. 2004. Influence of Age and Production Type on Liver Copper Concentrations in Calves. Journal of Veterinary Diagnostic Investigation. 16:382–387. doi:10.1177/104063870401600503.

Rademacher, R. D., S. Buczinski, H. M. Tripp, M. D. Edmonds, and E. G. Johnson. 2013. Systematic thoracic ultrasonography in acute bovine respiratory disease of feedlot steers. Bov Pract (Stillwater). 48:1–10. doi:10.21423/bovine-vol48no1p1-10.

Rivera, J. D., G. C. Duff, M. L. Galyean, D. A. Walker, and G. A. Nunnery. 2002. Effects of supplemental vitamin E on performance, health, and humoral immune response of beef cattle 1,2. J. Anim. Sci. 80:933–941. Available from: https://academic.oup.com/jas/article/80/4/933/4789390

Sacco, R. E., J. L. Mcgill, M. V Palmer, J. D. Lippolis, T. A. Reinhardt, and B. J. Nonnecke. 2012. Neonatal Calf Infection with Respiratory Syncytial Virus: Drawing Parallels to the Disease in Human Infants. Viruses. 4:3731–3753. doi:10.3390/v4123731. Available from: www.mdpi.com/journal/viruses

Slate, J. R., B. O. Chriswell, R. E. Briggs, and J. L. McGill. 2021. The Effects of Ursolic Acid Treatment on Immunopathogenesis Following Mannheimia haemolytica Infections. Front Vet Sci. 8. doi:10.3389/fvets.2021.782872.

Smedley, R., T. Mullaney, and W. Rumbeiha. 2009. Copper-Associated Hepatitis in Labrador Retrievers. Vet Pathol. 46:484–490. doi:10.1354/vp.08-VP-0197-S-FL.

Strickland, J. M., T. H. Herdt, D. G. Sledge, and J. P. Buchweitz. 2019. Short communication: Survey of hepatic copper concentrations in Midwest dairy cows. J Dairy Sci. 102:4209–4214. doi:10.3168/jds.2018-15566.

Theurer, M. E., J. Trent Fox, T. A. Portillo, N. F. Meyer, D. T. Ensley, and D. G. Renter. 2020. Comparison of gamithromycin post-treatment intervals for beef cattle naturally affected with bovine respiratory disease. Bov Pract (Stillwater). 54.

Turnlund, J. R., R. A. Jacob, C. L. Keen, J. Strain, D. S. Kelley, J. M. Domek, W. R. Keyes, J. L. Ensunsa, J. Lykkesfeldt, and J. Coulter. 2004. Long-term high copper intake: effects on indexes of copper status, antioxidant status, and immune function in young men. Am J Clin Nutr. 79:1037–1044. doi:10.1093/ajcn/79.6.1037.

Wang, X., J. R. Chowdhury, and N. R. Chowdhury. 2006. Bilirubin metabolism: Applied physiology. Current Paediatrics. 16:70–74. doi:10.1016/J.CUPE.2005.10.002.

Wood, K. M., S. I. Palmer, M. A. Steele, J. A. Metcalf, and G. B. Penner. 2015. The influence of age and weaning on permeability of the gastrointestinal tract in Holstein bull calves. J Dairy Sci. 98:7226–7237. doi:10.3168/jds.2015-9393.

Yamaguchi, M., and R. Yamaguchi. 1986. Action of zinc on bone metabolism in rats: Increases in alkaline phosphatase activity and DNA content. Biochem Pharmacol. 35:773–777. doi:10.1016/0006-2952(86)90245-5.