Journal of Animal Science, 2023, **101**, 1–14
https://doi.org/10.1093/jas/skad246
Advance access publication 19 July 2023
Animal Health and Well Being

Effects of wildfire smoke $PM_{2.5}$ on indicators of inflammation, health, and metabolism of preweaned Holstein heifers

Alexandra Pace, Patricia Villamediana, Pedram Rezamand, and Amy L. Skibiel*

Department of Animal, Veterinary and Food Sciences, University of Idaho, Moscow, ID 83844, USA
*Corresponding author: askibiel@uidaho.edu

Abstract

Wildfires are a growing concern as large, catastrophic fires are becoming more commonplace. Wildfire smoke consists of fine particulate matter ($PM_{2.5}$), which can cause immune responses and disease in humans. However, the present knowledge of the effects of wildfire $PM_{2.5}$ on dairy cattle is sparse. The present study aimed to elucidate the effects of wildfire-$PM_{2.5}$ exposure on dairy calf health and performance. Preweaned Holstein heifers ($N = 15$) were assessed from birth through weaning, coinciding with the 2021 wildfire season. Respiratory rate, heart rate, rectal temperatures, and health scores were recorded and blood samples were collected weekly or twice a week for analysis of hematology, blood metabolites, and acute phase proteins. Hourly $PM_{2.5}$ concentrations and meteorological data were obtained, and temperature–humidity index (**THI**) was calculated. Contribution of wildfires to $PM_{2.5}$ fluxes were determined utilizing AirNowTech Navigator and HYSPLIT modeling. Mixed models were used for data analysis, with separate models for lags of up to 7 d, and fixed effects of daily average $PM_{2.5}$, THI, and $PM_{2.5} \times$ THI, and calf as a random effect. THI ranged from 48 to 73, while $PM_{2.5}$ reached concentrations up to 118.8 μg/m³ during active wildfires. $PM_{2.5}$ and THI positively interacted to elevate respiratory rate, heart rate, rectal temperature, and eosinophils on lag day 0 (day of exposure; all $P < 0.05$). There was a negative interactive effect of $PM_{2.5}$ and THI on lymphocytes after a 2-d lag ($P = 0.03$), and total white blood cells, neutrophils, hemoglobin, and hematocrit after a 3-d lag (all $P < 0.02$), whereas there was a positive interactive effect on cough scores and eye scores on lag day 3 (all $P < 0.02$). Glucose and NEFA were increased as a result of combined elevated $PM_{2.5}$ and THI on lag day 1, whereas BHB was decreased (all $P < 0.05$). Contrarily, on lag day 3 and 6, there was a negative interactive effect of $PM_{2.5}$ and THI on glucose and NEFA, but a positive interactive effect on BHB (all $P < 0.03$). Serum amyloid A was decreased whereas haptoglobin was increased with elevated $PM_{2.5}$ and THI together on lag days 0 to 4 (all $P < 0.05$). These findings indicate that exposure to wildfire-derived $PM_{2.5}$, along with increased THI during the summer months, elicits negative effects on preweaned calf health and performance both during and following exposure.

Lay Summary

Wildfires contribute to fine particulate matter ($PM_{2.5}$) pollution throughout the United States. Wildfire-$PM_{2.5}$ exposure negatively affects human health and dairy cow production; however, the effects on calves are not known. We monitored preweaned calves exposed to natural wildfires to understand how wildfire-$PM_{2.5}$ exposure affects calf health and performance. Calves exposed to wildfire $PM_{2.5}$ and elevated temperature–humidity index (THI) experienced respiratory symptoms, alterations in blood cell composition and metabolism, and changes in circulating inflammatory proteins. These results suggest that $PM_{2.5}$ along with increased THI induced an inflammatory response and alterations in energy metabolism that may contribute to calf health and performance deficits.

Key words: acute phase response, air quality, dairy calf, hematology, inflammation

Abbreviations: BAS, basophils; BHB, β-hydroxybutyrate; EOS, eosinophils; HCT, hematocrit; HGB, hemoglobin; Hp, β-hydroxybutyrate; LYMPH, lymphocytes; MONO, monocytes; NEFA, β-hydroxybutyrate; NEU, neutrophils; NOAA, National Oceanic and Atmospheric Administration; PM, particulate matter; $PM_{2.5}$, fine particulate matter; RBC, red blood cells; SAA, β-hydroxybutyrate; THI, β-hydroxybutyrate; WBC, white blood cells

Introduction

Wildfires burn land and impose poor air quality in the surrounding regions. In the year 2021 alone, there were 58,985 wildfires that burned 7,125,643 acres in the United States (NIFC, 2021). Since the mid-1980's, there has been a notable change in wildfire trends, including a four-fold increase in the area burned by wildfires (Burke et al., 2021) and a 84-d increase in the length of the annual wildfire season (Westerling, 2016). These recent observations have largely been attributed to historical fire exclusion practices and land-use related interferences that caused fire deficits (Marlon et al., 2012; Harris and Taylor, 2015; Parks et al., 2015), as well as shifts in climatic variables, leading to changes in ecosystem dynamics that result in biomass accumulation and flammability (Westerling et al., 2006; Littell et al., 2009; Westerling, 2016; Hanan et al., 2021). Trends for greater wildfire risk and area burned are predicted to continue into the future (Abatzoglou and Williams, 2016), despite efforts of land resource managers to reduce extreme, catastrophic wildfires (Halofsky et al., 2020). The western United States disproportionately experiences large wildfires (EPA, 2016; Nagy et al., 2018) that burn a greater number of acres as compared to the eastern United States (Nowacki and Abrams, 2008; EPA, 2016).

Received May 13, 2023 Accepted July 15, 2023.

Western states also house more than 1.8 million dairy heifer calves that are retained in production systems as replacement animals (NASS, 2022).

Wildfire emissions contain air pollutants that can travel substantial distances beyond the region in which the smoke plumes originate (Damoah et al., 2004; Hung et al., 2021). These emissions include harmful gases such as carbon monoxide, hydrogen cyanide, ammonia, volatile organic compounds, polycyclic aromatic hydrocarbons, and particulate matter (PM), among others (Urbanski et al., 2008; Reisen et al., 2015; Sokolik et al., 2019). PM, particularly fine particulate matter ($PM_{2.5}$), is often considered one of the most potent and relevant components of wildfire smoke to health. $PM_{2.5}$ consists of particles less than 2.5 μm in aerodynamic diameter and wildfire-derived $PM_{2.5}$ can contribute to up to 50% of the total $PM_{2.5}$ pollution in the western United States (Kelly and Fussell, 2012; Burke et al., 2021). Furthermore, 90% of particulate mass in wildfire smoke is $PM_{2.5}$ (Vicente et al., 2013). Wildfire-derived $PM_{2.5}$ can also be more toxic than $PM_{2.5}$ originating from other sources (Wegesser et al., 2009; Aguilera et al., 2021b) and is the pollutant most often associated with health risk in humans because of its ability to access the deeper recesses of the lung (Black et al., 2017b).

In humans, a variety of respiratory issues have been associated with wildfire-$PM_{2.5}$ exposure, including exacerbations of asthma (Borchers Arriagada et al., 2019; Kiser et al., 2020), respiratory diagnoses (Liu et al., 2015) such as bronchitis and pneumonia (Hutchinson et al., 2018), mortality (Doubleday et al., 2020; Chen et al., 2021a; Liu et al., 2021; Ye et al., 2022), and cardiovascular ailments (Dennekamp et al., 2015; Kollanus et al., 2016; Navarro et al., 2019; Chen et al., 2021b). Wildfire smoke-related morbidities and mortality in humans are thought to be caused largely by inflammation, characterized by changes in biomarkers of innate immune function and the inflammatory response (Black et al., 2017b). We demonstrated for the first time that wildfire-$PM_{2.5}$ exposure, either independently, or in combination with elevated temperature–humidity index (**THI**), decreases milk production, alters metabolism, and induces an innate immune response involving changes in immune cell populations in the blood of dairy cows (Anderson et al., 2022). However, little information is available concerning consequences of wildfire $PM_{2.5}$ on calf health and metabolic physiology.

Calves may be more susceptible to inhaled particulates than mature animals, as their organ and immune systems are immature, similar to human infants and young children (Barrington and Parish, 2001). In humans, exposure to PM is more hazardous for the young and the elderly (Ignotti et al., 2010; Le et al., 2014; Liu et al., 2015; Aguilera et al., 2021a). Studies on rhesus macaques found that exposure to wildfire smoke during infancy induced long term effects including nasal epigenome alterations (Brown et al., 2022), reduced lung function, and immune dysregulation (Black et al., 2017a). Moreover, it is well-established that a calf's health early in life has long-term impacts on its future performance. For example, in a recent meta-analysis, Buczinski et al. (2021) found that dairy heifers diagnosed with respiratory disease at a young age have lower average daily gains, lower milk production in their first lactation, and higher mortality and culling rates. This has implications for future farm profitability as heifer calves are often maintained in the herd as replacement animals. Therefore, considering how dairy calves are affected by natural disasters, such as wildfires, is an important undertaking.

The aim of the present study was to provide a preliminary understanding of the effects of $PM_{2.5}$ from wildfire smoke on preweaned dairy calves. We hypothesized that calves naturally exposed to wildfire smoke $PM_{2.5}$ experience an inflammatory response, as evidenced by increases in acute phase proteins, including serum amyloid A (SAA) and haptoglobin (Hp), as well as hematological indices. Additionally, we hypothesized that health parameters and blood metabolites (e.g., glucose, nonesterified fatty acids [NEFA], and β-hydroxybutyrate [BHB] concentrations), would be negatively affected by inhalation of natural wildfire $PM_{2.5}$.

Materials and Methods

Preweaned calves

Animal procedures were approved by the University of Idaho Institutional Animal Care and Use Committee (protocol #IACUC-2019-39). Sixteen Holstein heifer calves at the University of Idaho Dairy Center were enrolled in the study from their births in July 2021 until weaning in September. Sample size was based on our previous study in dairy cows (Anderson et al., 2022) and by power analysis (alpha = 0.05, power set to 0.8, and effect size of 0.2). Calves were housed in individual hutches bedded with shavings in a barn that was fully enclosed by a roof and wall on three sides with the fourth side consisting of only half a wall. There were no sources of ventilation other than outside air flow directly into the barn, so calves were exposed to ambient air conditions, including wildfire smoke. Calves were given a first feeding of colostrum (3.8 liters) collected from cows on farm within 4 h of birth, and a second colostrum feeding (1.9 liters) within 12 h of birth. Calves were fed a combination of saleable and high SCC milk (2.8 liters) two times per day until weaning. Calves were provided ad libitum access to water and offered starter grain at 5 d of age and alfalfa at 45 d of age. Calves were weaned with two step-downs; the first step-down at 53 d of age to 2.8 liters of milk one time per day, and the second step-down to complete weaning at 60 d of age. During the study, one calf required treatment for gastrointestinal complications and was removed from the study, so the final sample size was 15 heifer calves. Two additional calves were treated for pinkeye with oxytetracycline following exposure to smoke, and these animals were retained in the study.

Environmental data

Hourly $PM_{2.5}$ concentrations (μg/m^3), ambient temperature (°C), and relative humidity (%), were obtained from a local monitoring station maintained by the Idaho Department of Environmental Quality, which was located 5.7 km from the Dairy Center. Detailed information regarding instrumentation and sampling can be found in Idaho DEQ, 2021. Our area is characterized topographically by rolling hills and wheat fields, with sparse trees and other structures or topographical features that block winds, thus $PM_{2.5}$ levels recorded at the monitoring station are a fairly accurate representation of $PM_{2.5}$ levels on farm. THI was calculated with the equation shown below (Dikmen et al., 2008):

$$THI = [1.8 \; x \; Temperature \; (°C)] - [0.550.0055 * Relative \; Humidity \; (\%)] \; x \; [1.8 \; x \; Temperature \; (°C) \; 26].$$

In order to ascertain if increases in $PM_{2.5}$ were derived from wildfires, maps with active wildfires and $PM_{2.5}$ concentrations were created using AirNow-Tech Navigator (airnow-tech.org), and air mass trajectories were visualized using the National Oceanic and Atmospheric Administration's (NOAA) HYSPLIT atmospheric transport and dispersion modeling system (Draxler and Hess 1997, 1998; Draxler, 1999; Stein et al., 2015). Three different atmospheric heights (50, 100, and 150 m) were used to map air mass trajectories over the previous 72 h. The modeling shows the origin of air masses, active wildfires, and trajectories of $PM_{2.5}$ dispersion.

Calf exposure to wildfire $PM_{2.5}$ was determined following the parameters previously described in Anderson et al., (2022): 1) 24 h average $PM_{2.5}$ concentrations were above 35 $\mu g/m^3$, the U.S. EPA recommended threshold for human outdoor exposure to $PM_{2.5}$ (EPA, 2019), and 2) AirNow-Tech Navigator $PM_{2.5}$ and wildfire maps plus HYSPLIT wind trajectories showing wildfire smoke and $PM_{2.5}$ blowing from active wildfires to the location where calves were housed.

Health parameters

All calf measurements and blood samples were taken weekly prior to and following smoke exposure and twice weekly during smoke events (based on the exposure criteria detailed above), capturing baseline (i.e., before smoke, 3 d), during wildfire smoke exposure (3 d), and after smoke exposure (4 d), which resulted in 10 total sample days. Thus, individual animals served as their own controls. Health scoring was performed according to the University of Wisconsin-Madison Calf Health Scoring system (McGuirk, 2008). This scoring system accounts for various indicators of clinical health parameters and scores them each on a scale of zero (indicating normal status) to three (indicating clinical abnormalities). Parameters observed included nasal secretions (0 = normal or no discharge to 3 = large amount of bilateral discharge), eye secretions (0 = normal or no discharge to 3 = large amount of discharge), ear droopiness/position (0 = normal to 3 = head tilt, two ears drooping), induced and uninduced coughing (0 = neither induced nor spontaneous cough to 3 = repetitive uninduced coughing), fecal consistency (0 = normal to 3 = watery, soaks through shavings), joint (0 = normal, no inflammation to 3 = inflammation, pain, heat) and naval inflammation (0 = normal, no inflammation to 3 = inflammation, pain, heat, discharge). Respiratory rate (breaths/min) was measured at 1200 hours by counting the number of flank movements in one min. Rectal temperatures were recorded at 1230 hours using a calf rectal thermometer. Heart rates were measured at 1230 hours by placing a stethoscope cranially on the left side of the calf behind the elbow and counting the number of heart beats in one min.

Blood sampling

Ten milliliters of blood was collected via jugular venipuncture into sodium-heparin plasma tubes and serum tubes with clot-activator gel. Throughout the study, blood was collected immediately following the morning feeding. Tubes were gently inverted several times immediately after collection, plasma tubes were placed on ice, and serum tubes were allowed to remain undisturbed at room temperature for approximately 30 min. A small volume of whole blood was used for hematology analysis prior to centrifugation at 3,000 × *g* for 17 min at 24 °C. Serum and plasma samples were aliquoted into Eppendorf tubes and stored at –20 °C until further analysis.

Hematology and acute phase proteins

A VetScan HM5C Hematology System (Abaxis, Union City, CA) was used to measure total and differential cell counts, concentrations of total white blood cell population (WBC), lymphocytes (LYMPH), monocytes (MONO), neutrophils (NEU), eosinophils (EOS), basophils (BAS), red blood cells (RBC), as well as hemoglobin (HGB) and hematocrit (HCT), expressed as a percentage. Following manufacturer procedure, approximately 2 mL of whole blood was placed in the sample receiving chamber, and samples were run using the automated bovine hematology reference intervals provided by the manufacturer.

SAA was measured in calf serum through immuno-absorbance with a SAA multispecies solid phase sandwich ELISA following the manufacturer's protocol (no. TP-802, Tridelta Development Ltd, Kildare, Ireland). Samples were analyzed in duplicate with a Spectramax ID3 spectrophotometer (Molecular Devices, San Jose, California), and diluted from 1:500 to 1:1,500 as necessary. Inter-assay CV was 12%, and intra-assay CV was 4.8%. Serum Hp concentrations were measured through an in-house kinetic colorimetric assay modified from U.S. patent US6451550B1 and previously utilized by our group (Tsai, 2021) in which Hp is quantified through peroxidase activity as a result of the interaction with methemoglobin. Samples were run in triplicate and absorbance was read at 390 nm every min for 6 min. Intra-assay CV was 8.7%, and inter-assay CV was 2.4%.

Blood metabolites

Blood metabolites, including □-hydroxybutyrate (BHB), glucose, and NEFA, were quantified from a subset of samples from seven dates that spanned changes in $PM_{2.5}$ concentrations. Plasma BHB concentration was detected through an in-house assay that uses the enzyme 3-□-dehydrogenase to oxidize 3-BHB to acetoacetate, which concomitantly causes NAD+ to reduce to NADH, whose concentration is proportional to the concentration of 3-BHB (Tsai, 2021). The concentration of NADH was measured with a Spectramax spectrophotometer (Molecular Devices). Following addition of buffer (33 µg/mL 3-□-dehydrogenase (Roche no. 3HBDB-RO), 0.1M Tris, 0.1 M oxalic acid, 2mM EDTA, 2.25 mM NADH) to each sample or standard calibrator (no. 421-73791, FujiFilm Medical Systems, Lexington, MA), plates were incubated at 37 °C for 1 min and then read at 340 nm at 0, 1, and 2 min. NADH concentrations in samples and standard were measured in triplicate. Inter-assay CV was 8.75%, intra-assay CV was 6.32%.

Concentrations of plasma NEFA and glucose were both measured using commercial kits. Glucose concentration was measured in duplicate with a microtiter assay (Fujifilm Autokit Glucose, cat 99703001) following the manufacturer protocol. Inter-assay CV was 14.3% and intra-assay CV was 7.2%. Plasma NEFA concentration was quantified in duplicate utilizing a commercial colorimetric Wako NEFA-HR assay kit (no's: Color reagent A: 999-34691; Solvent A: 995-34791; Color reagent B: 991-34891; Solvent B: 993-35191; NEFA Standard Solution: 276-76491; FUJIFILM Medical Systems) in accordance with the manufacturer's protocol. Intra-assay CV was 5.8%, and inter-assay CV was 7.6%.

Statistical analysis

Statistical analysis was performed in SAS version 9.4. Correlation analysis using the CORR procedure was used to

determine if $PM_{2.5}$, THI, or animal age were correlated. Age and $PM_{2.5}$ were not significantly correlated but $PM_{2.5}$ and THI were. PROC REG was used to determine bias in estimate coefficients and standard errors due to collinearity among independent variables by assessing tolerance (TOL), variance inflation factors (VIF), and condition indices (COLLIN). TOL values below 0.1 indicate multicollinearity (Schreiber-Gregory and Jackson, 2017). VIF above 10 are indicative of biases in standard errors due to multicollinearity (Myers, 1990) and COLLIN above 30 indicate strong bias whereas values between 5 and 10 indicate weak bias in coefficient estimates due to multicollinearity (Belsley et al., 1980).

General linear mixed models using the PROC MIXED procedure were used for all variables except health scores. Health scores were analyzed with logistic regression models using PROC GENMOD. In all models, fixed effects were $PM_{2.5}$, THI, and their interaction, while calf was a random effect. $PM_{2.5}$ and THI interactions that were not significant were removed from final models. Separate models with lags up to 7 d were utilized to describe both delayed and persistent effects. Plotted residuals were assessed for normality. Significance was considered when *P*-values were less than or equal to 0.05, whereas tendencies were considered when *P*-values were between 0.05 and 0.1. All data are presented as means ± SEM.

Results

Environmental data

$PM_{2.5}$ fluctuated with nearby fire conditions for the duration of the study, with a large smoke event occurring in mid-August when daily $PM_{2.5}$ concentrations surpassed 115 µg/m^3 (Figures 1 and 2). The 24-h average THI ranged from 48 to 73, while $PM_{2.5}$ concentrations ranged from 2.58 to 118.8 µg/m^3. During our study, the criteria for calf exposure to wildfire $PM_{2.5}$ were met on a total of three sample days and there were 10 total sample days, indicated in Figure 1. Thus, there were three calf measurements before, three calf measurements during, and four measurements taken after wildfire-$PM_{2.5}$ exposures. PM was moderately positively correlated with THI ($r = 0.47$, $P < 0.001$). TOL values were above 0.7, VIF were less than 1.4, and COLLIN were less than 3, indicating little to no bias in coefficient estimates or standard errors due to collinearity between $PM_{2.5}$ and THI.

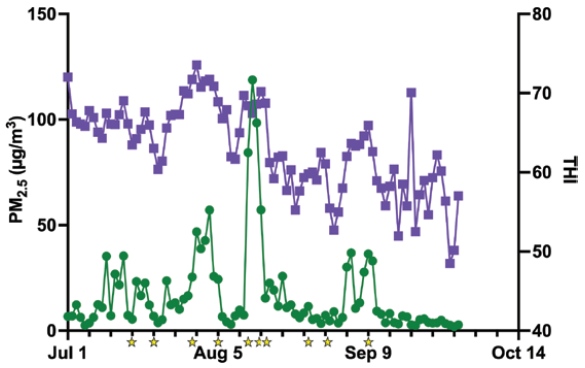


Figure 1. Average daily $PM_{2.5}$ concentrations and THI from July 1 to October 1, 2021, which encompassed the duration of the study period. There were multiple wildfire events over the course of the study period, resulting in spikes in daily average $PM_{2.5}$. Purple squares indicate THI and green circles represent $PM_{2.5}$. Yellow stars below the x-axis indicate dates in which samples were collected.

Health parameters

Wildfire smoke $PM_{2.5}$ and THI had interacting effects on several measures of health in calves (Figure 3, Table 1). There was a positive interacting effect of $PM_{2.5}$ and THI on rectal temperature on lag day 0 ($P = 0.04$), lag day 6 ($P = 0.03$), and lag day 7 ($P < 0.0001$). Elevated $PM_{2.5}$ and THI together also increased heart rate on lag day 0 ($P < 0.001$) and lag day 1 ($P < 0.0001$), and decreased heart rate on lag day 3 ($P < 0.0001$), lag day 6 ($P < 0.01$), and lag day 7 ($P < 0.001$). Respiratory rate was altered as a result of increased $PM_{2.5}$ and THI, increasing on lag day 0 ($P = 0.04$) but decreasing on lag day 2 ($P < 0.01$), lag day 3 ($P < 0.0001$), and lag day 5 ($P = 0.05$). Additionally, there was a tendency for higher THI and $PM_{2.5}$ together to increase respiratory rate on lag day 1 ($P = 0.06$), heart rate on lag day 4 ($P = 0.1$), and rectal temperature on lag day 3 ($P = 0.09$). Higher THI alone increased respiratory rate on lag day 4 ($P = 0.0001$) and on lag day 6 ($P = 0.09$). Health scores were affected by the interaction of elevated of $PM_{2.5}$ and THI (Table 2). Cough score ($P < 0.01$) and eye score ($P = 0.01$) were increased on lag day 3, but eye score decreased on lag day 4 ($P = 0.04$) with combined elevated $PM_{2.5}$ and THI. Additionally, the interaction of increased $PM_{2.5}$ and THI had a tendency to increase cough scores on lag day 7 ($P = 0.10$).

Hematology and acute phase proteins

Multiple hematological variables were affected by exposure to increased $PM_{2.5}$ and THI, most frequently on lag day 3 (Figure 4, Table 3). $PM_{2.5}$ and THI together decreased WBC ($P = 0.01$) and NEU counts ($P < 0.01$) on lag day 3. Together, $PM_{2.5}$ and THI interacted to decrease LYMPH on lag day 2 ($P = 0.03$), and increased EOS on lag day 0 ($P = 0.02$). We detected no effect of THI, $PM_{2.5}$, or their interaction on BAS or MONO. Elevated THI and $PM_{2.5}$ interacted to increase HCT on lag day 1 ($P < 0.01$), and to decrease HCT on lag day 3 ($P < 0.0001$) and lag day 5 ($P = 0.03$). HGB was also decreased on lag day 3 ($P < 0.0001$) and lag day 5 ($P < 0.01$) as a result of the interaction of elevated $PM_{2.5}$ and THI. Together, elevated $PM_{2.5}$ and THI also slightly increased NEU ($P = 0.06$) and RBC ($P = 0.07$) on lag day 1 and decreased EOS on lag day 3 ($P = 0.08$).

While not a main objective of the present study, we observed THI alone had an effect on several hematological variables. Neutrophil counts were increased by higher THI on lag day 0 ($P < 0.01$), while EOS were decreased on lag day 1 ($P = 0.04$). THI had a positive effect on HCT and HGB on lag days 0, 2, 4, and 6 (all $P < 0.04$). Hemoglobin concentration was also increased by higher THI on lag day 1 ($P < 0.01$). Red blood cells were increased by elevated THI on lag day 3 ($P = 0.03$). Additionally, THI tended to increase NEU on lag day 2 ($P = 0.07$) and lag day 5 ($P = 0.06$) and decrease EOS on lag day 6 ($P = 0.06$).

Higher $PM_{2.5}$ and THI together changed circulating SAA and Hp concentrations (Table 4). On lag days 0, 2, 3, and 4, SAA was decreased while Hp was increased (all $P < 0.05$) with higher combined $PM_{2.5}$ and THI. However, on lag day 1, SAA was increased while Hp was decreased (both $P < 0.01$) with higher combined $PM_{2.5}$ and THI. Additionally, elevated

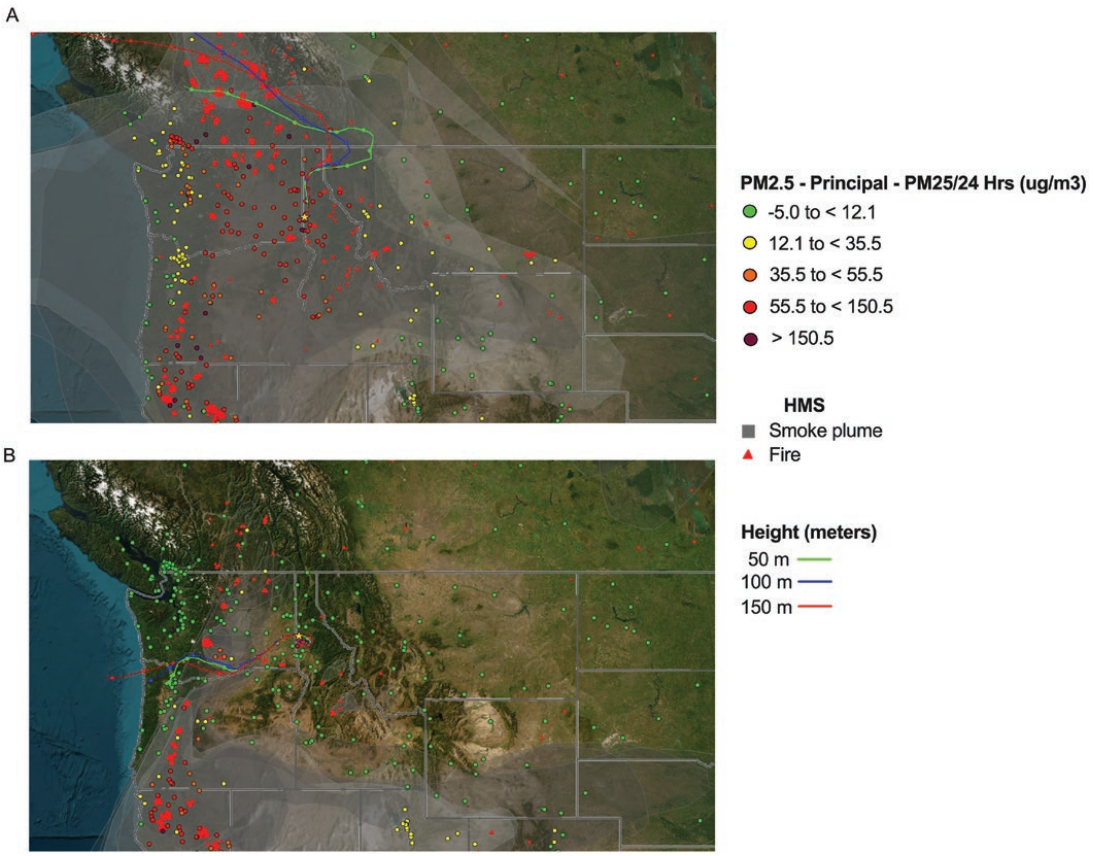


Figure 2. Maps of active wildfires, $PM_{2.5}$, and air mass trajectories on two separate days. (A) A day with wildfire smoke present (August 13, 2021), resulting in a 24-h average $PM_{2.5}$ concentration of 118.75 μg/m^3. (B) A day without wildfire smoke present (August 29, 2021), resulting in 24-h average $PM_{2.5}$ concentration of 3.42 μg/m. AirNow-Tech Navigator (https://www.airnowtech.org) mapping system was used to produce the maps, with NOAA HYSPLIT atmospheric transport and dispersion modeling system utilized to visualize air mass trajectories. Air masses are shown as 72-h backward trajectories, at atmospheric heights of 50, 100, and 150 m (green, blue, and red lines, respectively). Colored dots indicate ranges of $PM_{2.5}$ concentrations at monitoring stations. HMS indicates Hazard Mapping System, with smoke plumes and wildfire locations displayed. The yellow star depicts the location of the University of Idaho Dairy Center where the research was conducted.

THI and $PM_{2.5}$ had a positive interactive effect on SAA on lag day 7 ($P < 0.001$), and a negative interactive effect on Hp on lag day 5 ($P < 0.01$). Hp was decreased with increasing THI alone on lag day 7 ($P = 0.04$).

Blood metabolites

Elevated $PM_{2.5}$ and THI had an interactive effect on blood glucose, BHB, and NEFA concentrations, largely on lag days 1, 3, and 6 (Table 5). The effect on each metabolite on lag day 1 was opposite of that observed on subsequent lag days. Glucose was increased on lag day 1 ($P < 0.01$) and decreased on lag day 3 ($P = 0.02$) and lag day 6 ($P < 0.001$) with combined elevated $PM_{2.5}$ and THI. Also, there was a negative interacting effect of $PM_{2.5}$ and THI on BHB on lag day 1 ($P < 0.001$) and a positive interacting effect on lag day 3 ($P < 0.001$), day 6 ($P < 0.0001$), and day 7 ($P < 0.01$). Additionally, NEFA was increased on lag day 1 ($P = 0.05$) and decreased on lag day 3 ($P < 0.001$), day 6 ($P < 0.0001$), and day 7 ($P = 0.03$) when $PM_{2.5}$ and THI together were increased.

Higher THI alone affected blood metabolites on several days. Glucose increased with THI on lag day 0, ($P < 0.001$), day 2 ($P < 0.0001$), day 5 ($P < 0.01$), and day 7 ($P < 0.001$). BHB was decreased as a result of increased THI on lag day 2 ($P < 0.01$) and lag day 5 ($P = 0.04$), while NEFA was increased with higher THI on lag day 2 ($P < 0.01$) and on lag day 5 ($P = 0.04$). There was also a tendency for THI alone to increase glucose on lag day 4 ($P = 0.09$) and decrease BHB on lag day 0 ($P = 0.08$) and lag day 4 ($P = 0.1$).

Discussion

Wildfires cause escalations in atmospheric $PM_{2.5}$ that far exceed the threshold for hazardous exposure in humans. Further, projection models show that wildfires and as a consequence, air pollution associated with wildfire smoke, will continue to worsen over the next several decades (Littell et al., 2010). Wildfire-derived $PM_{2.5}$ inhalation in humans is linked to respiratory morbidities (Liu et al., 2015; Hutchinson et al., 2018; Borchers Arriagada et al., 2019; Kiser et al., 2020), cardiovascular disease (Dennekamp et al., 2015; Kollanus et al., 2016; Navarro et al., 2019; Chen et al., 2021b), and mortality (Doubleday et al., 2020; Liu et al., 2021; Chen et al., 2021a; Ye et al., 2022). Recently, we reported that lactating dairy cows naturally exposed to wildfire smoke, which produced daily average $PM_{2.5}$ concentrations reaching roughly 300 μg/m^3, experienced changes in milk production and metabolism and had an innate immune response (Anderson et al., 2022). However, impacts of wildfire smoke on calves had not yet

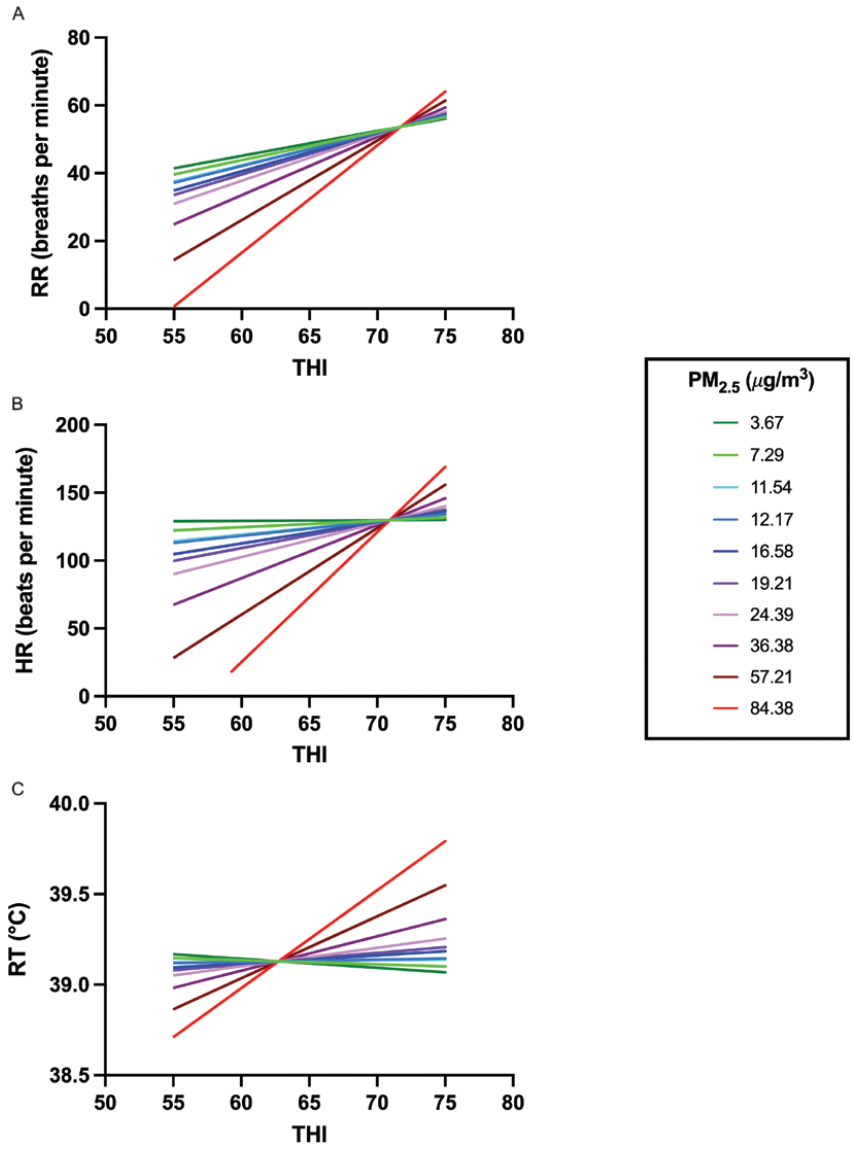


Figure 3. Interactions between fine particulate matter ($PM_{2.5}$) and temperature–humidity index (THI) at lag day 0 on (A) calf respiratory rate (RR), (B) heart rate (HR), and (C) rectal temperature (RT). Preweaned Holstein heifer calves ($N = 15$) born in July of 2021 were naturally exposed to wildfire smoke multiple times throughout the summer. Respiratory rate was increased as a result of $PM_{2.5}$ and THI interactions ($P = 0.04$). $PM_{2.5}$ and THI had a positive interaction on heart rate ($P < 0.001$). Rectal temperature was increased due to the positive interaction of $PM_{2.5}$ and THI ($P = 0.04$).

been investigated. Herein, we evaluated preweaned Holstein heifer calves naturally exposed to wildfire smoke to explore effects on calf health and performance.

Physiological measurements and health scores

In the present study, we found that when both wildfire $PM_{2.5}$ and THI increased, there was a concomitant increase in calf heart rate, respiratory rate, and rectal temperature. This supports a wide body of literature on the effects of wildfire smoke exposure on human cardiac and pulmonary systems. For example, heart rhythm disturbances and higher heart rate in humans have been previously associated with $PM_{2.5}$ from wood smoke or wildfire smoke (Unosson et al., 2013; Le et al., 2014). Additionally, heat stressed calves have higher heart rates, respiratory rates, and rectal temperatures (Kovács et al., 2018; Dado-Senn et al., 2020). The thermoregulatory role of respiration may pose increased risk to calves that are subject to both higher temperatures and pollution from smoke as there may be an increase in rate of inhalation of PM. A similar observation has been made in rats exposed to pollutants while exercising (Mautz, 2003). Thus, animals concomitantly exposed to high THI resulting in elevated body temperature and higher respiratory rate, could be more vulnerable to toxic compounds in wildfire smoke.

Both eye scores and cough scores were increased 3 d after calf exposure to elevated wildfire $PM_{2.5}$ and THI together. Ocular discharge following exposure could indicate irritation of the eye, respiratory tract, or both. Indeed, eye irritation has previously been reported in children exposed to wildfire

Table 1. Physiological measurements on lag day 0 as related to atmospheric $PM_{2.5}$ and THI

Model predictors												
Variable [1]	Intercept			$PM_{2.5}$, μg/m³			THI			$PM_{2.5}$ × THI		
	β	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value
RR[a]	9.12	15.3	–	−2.17	1	0.03	0.62	0.24	0.01	0.03	0.02	0.04
HR[b]	157.1	33.9	–	−8.4	2.2	<0.001	−0.38	0.52	0.46	0.12	0.03	<0.001
RT[c]	39.6	0.37	–	−0.05	0.02	0.06	−0.01	< =0.01	0.18	0.0007	0.0004	0.04

[1]RR = Respiratory rate (breaths per min); HR = heart rate (beats/min); RT = rectal temperature (°C).
[a]Elevated $PM_{2.5}$ and THI also interacted to decrease RR on lag day 2 ($P < 0.01$), lag day 3 ($P < 0.0001$), and lag day 5 ($P = 0.05$). Higher THI alone increased RR on lag day 4 ($P = 0.0001$).
[b]Elevated $PM_{2.5}$ and THI also interacted to increase HR on lag day 1 and decrease HR on lag day 3 (both $P < 0.0001$), lag day 6 ($P < 0.01$), and lag day 7 ($P < 0.001$).
[c]Elevated $PM_{2.5}$ and THI also interacted to increase RT on lag day 6 ($P = 0.03$) and lag day 7 ($P < 0.0001$).
Preweaned Holstein heifer calves ($N = 15$) born in July 2021 were naturally exposed to several wildfire smoke events across the summer. Data were analyzed using general linear mixed models with lags of up to 7 d, with $PM_{2.5}$, THI, and their interaction as fixed effects, and calf as a random effect. Coefficients, standard error of the means (SEM), and *P*-values from statistical models are provided.

Table 2. Calf health scores on lag day 3 in relation to atmospheric $PM_{2.5}$ and THI

Model predictors												
Variable [1]	Intercept [2]			$PM_{2.5}$,μg/m³			THI			$PM_{2.5}$ × THI		
	β	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value
Cough	2.84, 5,73, 7,38	3.78, 3.63, 3.60	–	−0.62	0.19	0.001	−0.12	0.06	0.001	0.01	0.003	0.001
Eye	14.72, 17.1	4.16, 4.14	–	−0.72	0.30	0.02	−0.29	0.06	<0.0001	0.01	0.004	0.01

[1]Cough = cough score; Eye = eye score.
[2]For cough scores, there were three intercepts, and for eye scores there were two intercepts.
Preweaned Holstein heifer calves ($N = 15$) born in July 2021 were naturally exposed to several wildfire smoke events across the summer. Data were analyzed using general linear mixed models with lags of up to 7 d, with $PM_{2.5}$, THI, and their interaction as fixed effects, and calf as a random effect. Coefficients, standard error of the means (SEM), and *P*-values from statistical models are provided.

smoke (Künzli et al., 2006). Coughing assists in the removal of particulates from the airways (Ackermann et al., 2010). Additionally, in the respiratory tract, mucociliary clearance and alveolar macrophages, both of which are involved in pulmonary particulate clearance (Ackermann et al., 2010), may be damaged by various smoke components, including PM (Ferreira-Ceccato et al., 2011; Franzi et al., 2011; Hamon et al., 2018). Thus, animals may cough in effort to assist compromised clearance mechanisms in removal of particulates and products of inflammation from their airways. Environmental pollutants, such as PM from dust on farms, are also related to incidence of BRD and pulmonary consolidation in calves (Dubrovsky et al., 2019; van Leenen et al., 2021). Although not evaluated in the present study, inflammation and compromised integrity of pulmonary tissues from PM could alter the pleural environment in a manner such that opportunistic pathogens could cause secondary bacterial infections. This etiology could also result in the clinical signs we observed such as coughing, ocular discharge, and elevated rectal temperatures.

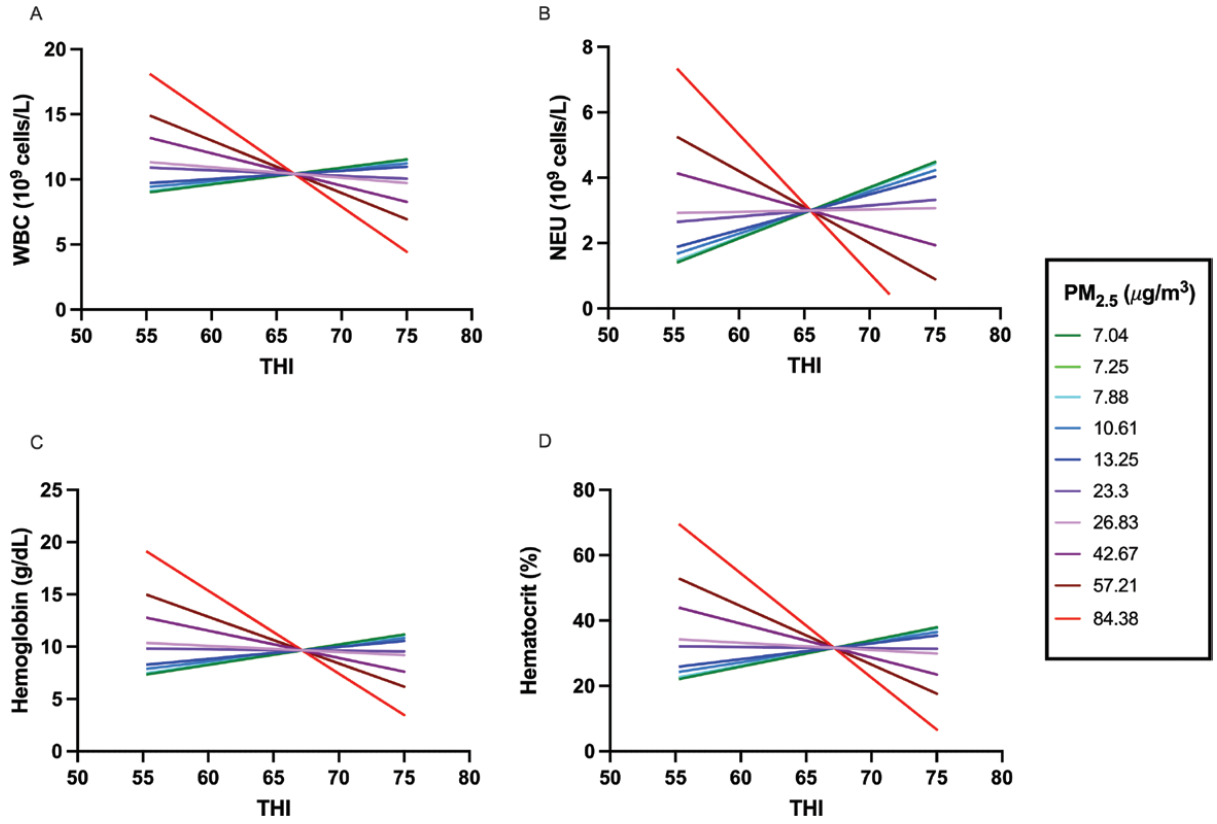
Hematology

In the present study, we observed delayed reductions in several white blood cell populations in systemic circulation. There was a negative interacting effect of elevated wildfire $PM_{2.5}$ and THI on LYMPH after 2 d, and on WBC and NEU after 3 d. These findings are similar to those described in lactating dairy cows, which had lower lymphocytes, neutrophils, and total white blood cell counts following exposure to higher $PM_{2.5}$ and THI (Anderson et al., 2022). Previous studies using other animal models have reported similar reductions in leukocytes in blood including lower total white blood cells and lymphocytes of mice exposed to smoke-derived PM (Kim et al., 2018), as well as delayed reductions in lymphocytes in the lung (Ramos et al., 2021) following exposure to PM.

Leukocyte recruitment to the respiratory tract could explain the overall reduced white blood cell counts in circulation. For example, in cattle, cells such as alveolar macrophages release chemokines after being activated by cytokines, which allows them to recruit leukocytes to the lung in response to pulmonary insult (Ackermann et al., 2010). Inflammatory responses related to secondary infections by opportunistic pathogens, or even pathogens carried by inhaled particulates, could also explain the delayed reduction in lymphocytes and neutrophils. In addition, stress caused by exposure to smoke may induce an elevation in circulating glucocorticoids, which can result in lymphocyte depletion as well as impairment of neutrophil function (Roth and Kaeberle, 1982; Latimer, 2011); however, circulating glucocorticoid concentration was not measured in our study.

We also observed an increase in eosinophils on the day of exposure to higher $PM_{2.5}$ and THI together. Bovine eosinophilia is typically associated with parasitic and hypersensitivity responses, such as in Th2 allergic response (Ackermann et al., 2010). Because a parasite control program was employed on farm and the response we observed was relatively immediate and transient, it is likely that the increase in circulating eosinophils was a result of a hypersensitivity response.

Figure 4. Interactions between $PM_{2.5}$ and THI at lag day 3 on (A) total white blood cell count, (B) neutrophil count, (C) hemoglobin concentration, and



(D) hematocrit. Preweaned Holstein heifer calves ($N = 15$) born in July 2021 were naturally exposed to wildfire smoke multiple times throughout the summer. Total white blood cell count was reduced after exposure to elevated THI and $PM_{2.5}$ together ($P = 0.01$). $PM_{2.5}$ and THI interacted to decrease neutrophil count ($P < 0.01$). Hemoglobin concentration was decreased as a result of the interaction of $PM_{2.5}$ and THI on lag day 3 ($P < 0.0001$). Combined increased $PM_{2.5}$ and THI resulted in lower hematocrit ($P < 0.0001$).

Elevated eosinophils following natural exposure to wildfire smoke has been previously described in dolphins (Venn-Watson et al., 2013) and in lactating dairy cows (Anderson et al., 2022).

As a result of exposure to combined higher $PM_{2.5}$ and THI, we found a transient increase of HCT and subsequently, a reduction in both HCT and HGB. It is unclear whether the initial polycythemia is relative or absolute, but could suggest reduced hydration in the calves, where there is loss of fluid volume resulting in relative polycythemia (Latimer, 2011; Roland et al., 2014). Reduced hydration impacts optimal mucociliary clearance, which can leave calves susceptible to amassing both pathogens and PM (Ackermann et al., 2010). However, higher hematocrit also could indicate increased oxygenation capacity of the blood, which could be a response to greater oxygenation demands of extravascular tissue or hypoxia. In this case, absolute polycythemia could be caused by higher metabolic rate, immune demands, or compromised respiratory function (Jones and Allison, 2007).

The subsequent reduction in HGB and HCT could be a reflection of the lower red blood cells per fluid volume proportionally decreasing hemoglobin concentrations; however, in the present study red blood cell counts were not reduced in calves during wildfire smoke exposure. Lower HGB and HCT could be indicative of greater water consumption, which could result in increased plasma volume (Roland et al., 2014). Alternatively, reduced HGB and HCT could indicate toxin-induced red blood cell lysis, which can cause hemolytic anemia, characterized by a reduction in hemoglobin and red blood cells in cattle (Roland et al., 2014). However, in the present study, clinical diagnosis of anemia was not confirmed. Anemia and reduced hemoglobin have been previously described in relation to ambient air pollution exposures including PM of various sizes (PM_{10}, $PM_{2.5}$, and $PM_{1.0}$) in humans (Nikolić et al., 2008; Morales-Ancajima et al., 2019; Elbarbary et al., 2020). Another study discovered a connection between anemia and pneumonia in children exposed to air pollution, indicating a possible role of anemia in respiratory outcomes during pollution exposure (Harris et al., 2011). Regardless of the etiology, decreased hemoglobin could indicate reduced oxygenation capacity to extravascular tissues.

In addition to $PM_{2.5}$, wildfire smoke contains PM_{10} that may also contribute to inflammatory responses in calves, including alterations in immune cell populations. For example, non-wildfire sources of PM_{10} in calf barns is associated with changes in neutrophil and epithelial cell percentages in broncho-alveolar fluid from dairy and beef calves (van Leenen et al., 2021). Although most of the PM fraction in wildfire smoke consists of $PM_{2.5}$ (Vicente et al., 2013),

Table 3. Calf blood hematology and inflammatory markers on lag day 3 in relation to atmospheric $PM_{2.5}$ and THI

Model predictors												
Variable [1]	Intercept			$PM_{2.5}$, μg/m³			THI			$PM_{2.5}$ × THI		
	β	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value
WBC	−3.08	4.85	–	0.71	0.28	0.01	0.2	0.08	0.01	−0.01	0.004	0.01
EOS[a]	−0.1	0.11	–	0.01	0.01	0.1	0.003	0.002	0.12	−0.0002	0.0001	0.08
BAS	0.02	0.05	–	−0.0002	0.0002	0.24	0.0004	0.0008	0.06	–	–	–
LYMPH[b]	6.25	2.98	–	0.24	0.17	0.17	0.002	0.05	0.96	−0.003	0.003	0.17
NEU[c]	−10.68	3.09	–	0.49	0.18	0.01	0.21	0.05	< 0.0001	−0.01	0.003	0.005
MONO	0.99	0.34	–	−0.0003	0.001	0.74	−0.004	0.01	0.49	–	–	–
RBC[d]	3.23	2.69	–	0.19	0.15	0.22	0.09	0.04	0.03	−0.003	0.002	0.19
HCT, %[e]	−46.6	7.12	–	3.47	0.41	<0.0001	1.17	0.11	<0.0001	−0.05	0.01	< 0.0001
HGB[f]	−9.45	1.57	–	0.86	0.09	< 0.0001	0.28	0.02	< 0.0001	−0.01	0.001	< 0.0001

[1]. Units for WBC, EOS, BAS, LYMPH, NEU, MONO are 10^9 cells/L; RBC is 10^{12} cells/L; HCT is %, and HGB is g/dL.
[a]Elevated $PM_{2.5}$ and THI also interacted to increase EOS on lag day 0 ($P = 0.02$). Higher THI alone decreased EOS on lag day 1 ($P = 0.04$).
[b]Elevated $PM_{2.5}$ and THI also interacted to decrease LYMPH on lag day 2 ($P = 0.03$).
[c]Elevated THI alone also increased NEU on lag day 0 ($P < 0.01$).
[d]Elevated THI alone also increased RBC on lag day 3 ($P = 0.03$).
[e]Elevated $PM_{2.5}$ and THI also interacted to decrease HCT on lag day 1 ($P < 0.01$) and lag day 5 ($P = 0.03$). THI alone increased HCT on lag day 0, lag day 2 (both $P < 0.0001$), lag day 4 ($P = 0.03$), and lag day 6 ($P < 0.001$).
[f]Elevated $PM_{2.5}$ and THI also interacted to decrease HGB on lag day 5 ($P < 0.01$). THI alone increased HGB on lag day 0 and lag day 2 (both $P < 0.0001$), lag day 1 ($P < 0.01$), lag day 4 ($P = 0.01$), and lag day 6 ($P < 0.01$).
Preweaned Holstein heifer calves ($N = 15$) born in July 2021 were naturally exposed to several wildfire smoke events across the summer. Data were analyzed using general linear mixed models with lags of up to 7 d, with $PM_{2.5}$, THI, and their interaction as fixed effects, and calf as a random effect. Coefficients, standard error of the means (SEM), and *P*-values from statistical models are provided.

Table 4. Calf acute phase proteins on lag days 0, 1, and 2 in relation to atmospheric $PM_{2.5}$ and THI

Model predictors												
Variable	Intercept			$PM_{2.5,}$ μg/m³			THI			$PM_{2.5}$ × THI		
	β	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value
Lag day 0												
Hp [a]	7.65	1.98	–	−1.94	0.61	<0.01	−0.08	0.03	<0.01	0.03	0.01	<0.01
SAA	−2,441,606	810,682	–	567,303	249,373	0.03	37,914	11,028	0.001	−8,104.37	3,541.01	0.03
Lag day 1												
Hp	−37.37	14.06	–	4.09	1.47	0.01	0.58	0.21	<0.01	−0.06	0.02	<0.01
SAA	15,249,213	5,764,383	–	−1,644,146	604,162	0.01	−222,767	85,609	0.01	23,988	8,821.01	0.01
Lag dAY 2												
Hp	41.71	13.44	–	−8.91	2.99	<0.01	−0.59	0.20	<0.01	0.132	0.04	<0.01
SAA	−1.2E + 07	5,493,039	–	2,561,002	1,223,081	0.04	183,978	80,870	0.03	−37,976	18,129	0.04

[a]Elevated $PM_{2.5}$ and THI together also increased Hp on lag day 3 ($P < 0.01$) and lag day 4 ($P < 0.03$), and decreased Hp on lag day 5 ($P < 0.01$). THI alone decreased Hp on lag day 7 ($P < 0.040$).
[b]Higher $PM_{2.5}$ and THI together also decreased SAA on lag day 3 ($P = 0.001$) and lag day 4 ($P < 0.0001$), and increased SAA on lag day 7 ($P < 0.001$).
Preweaned Holstein heifer calves ($N = 15$) born in July 2021 were naturally exposed to several wildfire smoke events across the summer. Data were analyzed using general linear mixed models with lags of up to 7 d, with $PM_{2.5}$, THI, and their interaction as fixed effects, and calf as a random effect. Coefficients, standard error of the means (SEM), and *P*-values from statistical models are provided.

exploration of other components of wildfire smoke in relation to calf health would be a worthy avenue of future research.

Blood metabolites

We observed a shift in blood metabolites following wildfire smoke and elevated THI exposure, characterized by an initial increase in circulating glucose and NEFA concentrations concomitant with decreased BHB, followed by a reduction in glucose and NEFA, and higher BHB concentrations across subsequent days. This shift in blood metabolites indicates changes in utilization of available energy sources when exposed to elevated THI and $PM_{2.5}$ together. Similarly, NEFA was increased in lactating dairy cows exposed to combined elevated $PM_{2.5}$ and THI (Anderson et al., 2022). Changes in metabolism may result from reduced feed intake, however, we did not observe a reduction in milk intake during exposure. Thus, the metabolic responses observed are likely a reflection of increased energy utilization as a result of physiological demands, such as mounting an immune response.

Lower blood glucose following exposure to smoke sources has been previously described in the literature. Rats exposed to peat smoke, and subsequently given a high-fat oral gavage, had reduced blood glucose concentration, suggesting that inhalation of smoke could induce dysregulation of glucose metabolism (Martin et al., 2018). Additionally, a variety of

Table 5. Blood metabolites on lag days 1, 3, and 6 in relation to atmospheric fine particulate matter ($PM_{2.5}$) and temperature–humidity index (THI)

Model predictors												
Variable 1	Intercept			$PM_{2.5}$, μg/m³			THI			$PM_{2.5}$ × THI		
	β	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value	*β*	SEM	*P*-value
Lag day 1												
Glucose[a]	140.6	61.4	–	−16.3	5.23	0.002	−0.38	0.91	0.68	0.24	0.076	<0.01
BHB[b]	−54.3	137.7	–	40.9	11.7	<0.001	3.67	2.03	0.07	−0.6	0.17	<0.001
NEFA[c]	0.29	0.19	–	−0.033	0.02	0.04	−0.002	0.003	0.44	0.0005	0.0002	0.048
Lag day 3												
Glucose	−338.2	97.2	–	10.9	4.67	0.02	6.9	1.5	<0.0001	−0.163	0.07	0.02
BHB	1140.7	211.4	–	−39.5	10.1	<0.001	−14.4	3.26	<0.0001	0.58	0.15	<0.001
NEFA	−0.88	0.29	–	0.049	0.014	<0.001	0.015	0.004	<0.001	−0.0007	0.0002	<0.001
Lag day 6												
Glucose	−282.6	92.3	–	42.7	12.2	< 0.001	5.65	1.41	0.0001	−0.59	0.17	< 0.001
BHB	1284.3	187.2	–	−132.8	24.7	< 0.0001	−15.7	2.85	< 0.0001	1.87	0.34	< 0.0001
NEFA	−1.16	0.26	–	0.16	0.034	< 0.0001	0.019	0.004	< 0.0001	−0.002	0.0005	< 0.0001

[1]Glucose, mg/dL; BHB, □–hydroxybutyrate, μM; NEFA, nonesterified fatty acids, mEq/L.
[a]Higher THI alone increased glucose on lag day 0 ($P < 0.001$), lag day 2 ($P < 0.0001$), lag day 5 ($P < 0.01$), and lag day 7 ($P < 0.001$).
[b]Higher PM_2.5 and THI also interacted to increase BHB on lag day 7 ($P < 0.01$). Higher THI alone decreased BHB on lag day 2 ($P < 0.01$) and lag day 5 ($P = 0.04$).
[c]Higher $PM_{2.5}$ and THI also interacted to decrease NEFA on lag day 7 ($P = 0.03$). Higher THI alone also increased NEFA on lag day 2 ($P < 0.01$) and lag day 5 ($P = 0.04$).
Preweaned Holstein heifer calves ($N = 15$) born in July 2021 were naturally exposed to several wildfire smoke events across the summer. Data were analyzed using general linear mixed models with lags of up to 7 d, with $PM_{2.5}$, THI, and their interaction as fixed effects, and calf as a random effect. Coefficients, standard error of the means (SEM), and *P*-values from statistical models are provided.

stressors, such as environmental changes, increased energy demands, or inflammation could directly influence nutrient metabolism. For instance, catecholamines and glucocorticoids, which can be elevated in response to environmental stressors such as heat stress (Farooq et al., 2010; W.S. Kim et al., 2018), respectively can induce lipolysis and thereby NEFA release, as well as gluconeogenesis, in cattle (Herdt, 2000; Hammon et al., 2012). Higher circulating pro-inflammatory cytokines are also linked to lipid metabolism, including elevated circulating NEFA concentrations (Kushibiki et al., 2002). Further research is needed to parse out the direct effects of exposure to concomitantly higher $PM_{2.5}$ and THI on feed intake and nutrient metabolism.

Acute phase proteins

Circulating Hp and SAA concentrations changed in opposite directions in response to calf exposure to combined elevated THI and $PM_{2.5}$. Hp and SAA are proteins involved in the acute phase response, which is part of the inflammatory response (Alsemgeest et al., 1994). An acute phase response following wildfire smoke exposure has been previously reported in humans and rats (Ferguson et al., 2016; Martin et al., 2018), as well as in rhesus macaques exposed in utero (Capitanio et al., 2022). While both Hp and SAA are positive acute phase proteins (Tothova et al., 2014), the oppositional changes in circulating Hp and SAA in calves in the present study suggest different roles in their responses to elevated $PM_{2.5}$ and THI. For example, Hp released from the liver and extrahepatic tissues, including the lung, binds to free HGB followed by clearance of Hp/HGB complexes (Yang et al., 1995), which may explain, in part, the reduced HGB detected in calves after exposure to elevated $PM_{2.5}$ and THI. This response can stymie oxidative damage produced by free hemoglobin (Yang et al., 2003).

SAA is involved in inflammatory responses by binding to lipids (Uhlar and Whitehead, 1999). Its involvement in lipid metabolism is particularly noteworthy here as we found that SAA was elevated simultaneously with higher NEFA on lag day 1. Guzelbektes et al. (2010) reported similar parallel elevations in both SAA and NEFA, which has been linked to the inflammatory mediator, TNF-α, response to lipid mobilization from adipocytes associated with negative energy balance in fresh cows (Ametaj et al., 2005). Additionally, because SAA can be used as an indicator of pneumonia (Ackermann et al., 2010; Joshi et al., 2018) and particularly in acute inflammation (Alsemgeest et al., 1994), the delayed elevation in SAA on lag day 7 could be the result of a secondary inflammatory response, which is also supported by our observations of delayed rectal temperature elevation that occurred at the same time as the increase in circulating SAA concentrations.

Conclusion

With the threat of wildfires continuing into the foreseeable future, the effects of hazardous emissions in smoke on production animal health are a growing welfare concern. Unlike humans, cattle are less likely to have access to shelter with ventilation that allows for protection from inhalation of smoke. Thus, many of the calves in the top states in dairy production may be at increased risk of exposure to wildfire smoke for the duration of a wildfire event. To our knowledge, this is the first study to investigate the effects wildfire smoke exposure has on preweaned calves. Our data indicate that calves experienced symptoms of respiratory irritation and clinical respiratory signs such as coughing and ocular discharge. This was combined with alterations in leukocyte counts and an acute phase response, indicative of a systemic innate immune response. Additionally, calf blood metabolites

shifted, suggesting changes in energy metabolism that may be associated with feed intake and/or increased energy requirements to support an immune response. Although we found delayed and persistent effects of wildfire smoke exposure on metabolism and the immune system, long-term effects and links between these physiological changes and calf morbidity and mortality have yet to be investigated..

Acknowledgments

We would like to thank NOAA Air Resources Laboratory (ARL) for access to HYSPLIT modeling resources and TANTUS Technologies, Inc. for use of AirNow-Tech Navigator utilized during this study. Meteorological data and $PM_{2.5}$ concentrations were obtained from the Idaho Department of Environmental Quality. Additionally, we are grateful for the support of the University of Idaho Dairy Center, Mallery Larson for technical support, Bruna Calvo Agustinho for guidance on laboratory assays, and Bill Price for his assistance on statistical analysis. This project was funded by the University of Idaho College of Agricultural and Life Sciences and the Department of Animal, Veterinary and Food Sciences.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

Literature Cited

Abatzoglou, J. T., and A. P. Williams. 2016. Impact of anthropogenic climate change on wildfire across western US forests. *Proc. Natl. Acad. Sci. U.S.A.* 113:11770–11775. doi:10.1073/pnas.1607171113

Ackermann, M. R., R. Derscheid, and J. A. Roth. 2010. Innate immunology of bovine respiratory disease. *Vet. Clin. North Am. Food Anim. Pract.* 26:215–228. doi:10.1016/j.cvfa.2010.03.001

Aguilera, R., T. Corringham, A. Gershunov, and T. Benmarhnia. 2021b. Wildfire smoke impacts respiratory health more than fine particles from other sources: observational evidence from Southern California. *Nat. Commun.* 12:1493. doi:10.1038/s41467-021-21708-0

Aguilera, R., T. Corringham, A. Gershunov, S. Leibel, and T. Benmarhnia. 2021a. Fine particles in wildfire smoke and pediatric respiratory health in California. *Pediatrics*. 147:e2020027128. doi:10.1542/peds.2020-027128

Alsemgeest, S. P. M., H. C. Kalsbeek, T. Wensing, J. P. Koeman, A. M. van Ederen, and E. Gruys. 1994. Concentrations of serum Amyloid-a (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle. *Vet. Q.* 16:21–23. doi:10.1080/01652176.1994.9694410

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci.* 85:165–175. doi:10.4141/a04-043

Anderson, A., P. Rezamand, and A. L. Skibiel. 2022. Effects of wildfire smoke exposure on innate immunity, metabolism, and milk production in lactating dairy cows. *J. Dairy Sci.* 105:7047–7060. doi:10.3168/jds.2022-22135

Barrington, G. M., and S. M. Parish. 2001. Bovine Neonatal Immunology. *Vet. Clin. North Am. Food Anim. Pract.* 17:463–476. doi:10.1016/s0749-0720(15)30001-3

Belsley, D. A., E. Kuh, and R. E. Welsch. 1980. *Regression diagnostics: identifying influential data and sources of collinearity*. New York, USA: John Wiley.

Black, C., J. E. Gerriets, J. H. Fontaine, R. W. Harper, N. J. Kenyon, F. Tablin, E. S. Schelegle, and L. A. Miller. 2017a. Early life wildfire smoke exposure is associated with immune dysregulation and lung function decrements in adolescence. *Am. J. Respir. Cell Mol. Biol.* 56:657–666. doi:10.1165/rcmb.2016-0380OC

Black, C., Y. Tesfaigzi, J. A. Bassein, and L. A. Miller. 2017b. Wildfire smoke exposure and human health: significant gaps in research for a growing public health issue. *Environ. Toxicol. Pharmacol.* 55:186–195. doi:10.1016/j.etap.2017.08.022

Borchers Arriagada, N., J. A. Horsley, A. J. Palmer, G. G. Morgan, R. Tham, and F. H. Johnston. 2019. Association between fire smoke fine particulate matter and asthma-related outcomes: systematic review and meta-analysis. *Environ. Res.* 179:108777. doi:10.1016/j.envres.2019.108777

Brown, A. P., L. Cai, B. I. Laufer, L. A. Miller, J. M. LaSalle, and H. Ji. 2022. Long-term effects of wildfire smoke exposure during early life on the nasal epigenome in rhesus macaques. *Environ. Int.* 158:106993. doi:10.1016/j.envint.2021.106993

Buczinski, S., D. Achard, and E. Timsit. 2021. Effects of calfhood respiratory disease on health and performance of dairy cattle: a systematic review and meta-analysis. *J. Dairy Sci.* 104:8214–8227. doi:10.3168/jds.2020-19941

Burke, M., A. Driscoll, S. Heft-Neal, J. Xue, J. Burney, and M. Wara. 2021. The changing risk and burden of wildfire in the United States. *Proc. Natl. Acad. Sci. U.S.A.* 118:e2011048118. doi:10.1073/pnas.2011048118.

Capitanio, J. P., L. A. Del Rosso, N. Gee, and B. L. Lasley. 2022. Adverse biobehavioral effects in infants resulting from pregnant rhesus macaques' exposure to wildfire smoke. *Nat. Commun.* 13:1774. doi:10.1038/s41467-022-29436-9

Chen, G., Y. Guo, X. Yue, S. Tong, A. Gasparrini, M. L. Bell, B. Armstrong, J. Schwartz, J. J. K. Jaakkola, A. Zanobetti, et al. 2021a. Mortality risk attributable to wildfire-related PM2·5 pollution: a global time series study in 749 locations. *Lancet Planet. Health*. 5:e579–e587. doi:10.1016/S2542-5196(21)00200-X

Chen, H., J. M. Samet, P. A. Bromberg, and H. Tong. 2021b. Cardiovascular health impacts of wildfire smoke exposure. *Part. Fibre Toxicol.* 18:2. doi:10.1186/s12989-020-00394-8

Dado-Senn, B., V. Ouellet, G. E. Dahl, and J. Laporta. 2020. Methods for assessing heat stress in preweaned dairy calves exposed to chronic heat stress or continuous cooling. *J. Dairy Sci.* 103:8587–8600. doi:10.3168/jds.2020-18381

Damoah, R., N. Spichtinger, C. Forster, P. James, I. Mattis, U. Wandinger, S. Beirle, T. Wagner, and A. Stohl. 2004. Around the world in 17 days – hemispheric-scale transport of forest fire smoke from Russia in May 2003. *Atmos. Chem. Phys.* 11:1311—1321. doi:10.5194/acp-4-1311-2004.

Dennekamp, M., L. D. Straney, B. Erbas, M. J. Abramson, M. Keywood, K. Smith, M. R. Sim, D. C. Glass, M. A. Del, A. Haikerwal, et al. 2015. Forest fire smoke exposures and out-of-hospital cardiac arrests in Melbourne, Australia: a case-crossover study. *Environ. Health Perspect.* 123:959–964. doi:10.1289/ehp.1408436

Dikmen, S., E. Alava, E. Pontes, J. M. Fear, B. Y. Dikmen, T. A. Olson, and P. J. Hansen. 2008. Differences in thermoregulatory ability between slick-haired and wild-type lactating Holstein cows in response to acute heat stress. *J. Dairy Sci.* 91:3395–3402. doi:10.3168/jds.2008-1072

Doubleday, A., J. Schulte, L. Sheppard, M. Kadlec, R. Dhammapala, J. Fox, and T. Busch Isaksen. 2020. Mortality associated with wildfire smoke exposure in Washington state, 2006–2017: a case-crossover study. *Environ. Health*. 19:4. doi:10.1186/s12940-020-0559-2

Draxler, R. R. 1999. HYSPLIT4 user's guide. NOAA Tech. Memo. ERL ARL-230. NOAA Air Resources Laboratory. https://www.arl.noaa.gov/wp_arl/wp-content/uploads/documents/reports/arl-230.pdf. Accessed January 21, 2021.

Draxler, R. R., and G. D. Hess. 1997. Description of the HYSPLIT_4 modeling system. NOAA Tech. Memo. ERL ARL-224. NOAA Air Resources Laboratory. https://www.arl.noaa.gov/documents/reports/arl-224.pdf. Accessed January 21, 2021.

Draxler, R. R., and G. D. Hess. 1998. An overview of the HYSPLIT_4 modeling system of trajectories, dispersion, and deposition. *Aust. Meteorol. Mag.* 47:295–308.

Dubrovsky, S. A., A. L. Van Eenennaam, B. M. Karle, P. V. Rossitto, T. W. Lehenbauer, and S. S. Aly. 2019. Epidemiology of bovine respiratory disease (BRD) in preweaned calves on California dairies: the BRD 10K study. *J. Dairy Sci*. 102:7306–7319. doi:10.3168/jds.2018-14774

Elbarbary, M., T. Honda, G. Morgan, Y. Guo, Y. Guo, P. Kowal, and J. Negin. 2020. Ambient air pollution exposure association with anaemia prevalence and haemoglobin levels in Chinese older adults. *Int. J. Environ. Res. Public Health*. 17:3209. doi:10.3390/ijerph17093209

EPA. 2016. Climate change indicators: wildfires. U.S. Environmental Protection Agency. https://www.epa.gov/climate-indicators/climate-change-indicators-wildfires. Accessed October 1, 2021.

EPA. 2019. Integrated Science Assessment (ISA) for Particulate Matter (Final Report, Dec. 2019). U.S. Environmental Protection Agency. https://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=347534. Accessed September 23, 2021.

Farooq, U., H. A. Samad, F. Shehzad, and A. Qayyum. 2010. Physiological responses of cattle to heat stress. *World Appl. Sci. J*. 8:38–43.

Ferguson, M. D., E. O. Semmens, C. Dumke, J. C. Quindry, and T. J. Ward. 2016. Measured pulmonary and systemic markers of inflammation and oxidative stress following wildland firefighter simulations. *J. Occup. Environ. Med*. 58:407–413. doi:10.1097/JOM.0000000000000688

Ferreira-Ceccato, A. D., E. M. C. Ramos, L. C. S. de Carvalho, R. F. Xavier, M.F. de S. Teixeira, P. A. Raymundo-Pereira, C. A. Proença, A. C. de Toledo, and D. Ramos. 2011. Short terms effects of air pollution from biomass burning in mucociliary clearance of Brazilian sugarcane cutters. *Respir. Med*. 105:1766–1768. doi:10.1016/j.rmed.2011.08.003

Franzi, L. M., J. M. Bratt, K. M. Williams, and J. A. Last. 2011. Why is particulate matter produced by wildfires toxic to lung macrophages? *Toxicol. Appl. Pharmacol*. 257:182–188. doi:10.1016/j.taap.2011.09.003

Guzelbektes, H., I. Sen, M. Ok, P. Constable, M. Boydak, and A. Coskun. 2010. Serum amyloid A and haptoglobin concentrations and liver fat percentage in lactating dairy cows with abomasal displacement. *J. Vet. Intern. Med*. 24:213–219. doi:10.1111/j.1939-1676.2009.0444.x

Halofsky, J. E., D. L. Peterson, and B. J. Harvey. 2020. Changing wildfire, changing forests: the effects of climate change on fire regimes and vegetation in the Pacific Northwest, USA. *Fire Ecol*. 16:4. doi:10.1186/s42408-019-0062-8

Hammon, H. M., J. Steinhoff-Wagner, U. Schönhusen, C. C. Metges, and J. W. Blum. 2012. Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. *Domest Anim. Endocrinol*. 43:171–185. doi:10.1016/j.domaniend.2012.02.005

Hamon, R., H. B. Tran, E. Roscioli, M. Ween, H. Jersmann, and S. Hodge. 2018. Bushfire smoke is pro-inflammatory and suppresses macrophage phagocytic function. *Sci. Rep*. 8:13424. doi:10.1038/s41598-018-31459-6

Hanan, E. J., J. Ren, C. L. Tague, C. A. Kolden, J. T. Abatzoglou, R. R. Bart, M. C. Kennedy, M. Liu, and J. C. Adam. 2021. How climate change and fire exclusion drive wildfire regimes at actionable scales. *Environ. Res. Lett*. 16:024051. doi:10.1088/1748-9326/abd78e

Harris, A. M., F. Sempértegui, B. Estrella, X. Narváez, J. Egas, M. Woodin, J. L. Durant, E. N. Naumova, and J. K. Griffiths. 2011. Air pollution and anemia as risk factors for pneumonia in ecuadorian children: a retrospective cohort analysis. *Environ. Health*. 10:93. doi:10.1186/1476-069X-10-93

Harris, L., and A. H. Taylor. 2015. Topography, fuels, and fire exclusion drive fire severity of the rim fire in an old-growth mixed-conifer forest, Yosemite National Park, USA. *Ecosystems*. 18:1192–1208. doi:10.1007/s10021-015-9890-9

Herdt, T. H. 2000. Ruminant adaptation to negative energy balance. *Vet. Clin. North Am. Food Anim. Pract*. 16:215–230, v. doi:10.1016/s0749-0720(15)30102-x

Hung, W. -T., S. Lu, S. Alessandrini, R. Kumar, and C. -A. Lin. 2021. The impacts of transported wildfire smoke aerosols on surface air quality in New York State: a multi-year study using machine learning. *Atmos. Environ*. 259:118513. doi:10.1016/j.atmosenv.2021.118513

Hutchinson, J. A., J. Vargo, M. Milet, N. H. F. French, M. Billmire, J. Johnson, and S. Hoshiko. 2018. The San Diego 2007 wildfires and Medi-Cal emergency department presentations, inpatient hospitalizations, and outpatient visits: an observational study of smoke exposure periods and a bidirectional case-crossover analysis. *PLoS Med*. 15:e1002601. doi:10.1371/journal.pmed.1002601

Idaho DEQ. 2021. Idaho department of environmental quality 2021 ambient air quality monitoring annual network plan. Idaho Department of Environmental Quality, Air Quality Division. 1–87. https://www2.deq.idaho.gov/admin/LEIA/index.html?view=-folder&id=1357

Ignotti, E., J. G. Valente, K. M. Longo, S. R. Freitas, S. de S. Hacon, and P. Artaxo Netto. 2010. Impact on human health of particulate matter emitted from burnings in the Brazilian Amazon region. *Rev. Saúde. Pública*. 44:121–130. doi:10.1590/s0034-89102010000100013

Jones, M. L., and R. W. Allison. 2007. Evaluation of the ruminant complete blood cell count. *Vet. Clin. North Am. Food Anim. Pract*. 23:377–402, v. doi:10.1016/j.cvfa.2007.07.002

Joshi, V., V. K. Gupta, A. G. Bhanuprakash, R. S. K. Mandal, U. Dimri, and Y. Ajith. 2018. Haptoglobin and serum amyloid A as putative biomarker candidates of naturally occurring bovine respiratory disease in dairy calves. *Microb. Pathog*. 116:33–37. doi:10.1016/j.micpath.2018.01.001

Kelly, F. J., and J. C. Fussell. 2012. Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. *Atmos. Environ*. 60:504–526. doi:10.1016/j.atmosenv.2012.06.039

Kim, W. S., J.-S. Lee, S. W. Jeon, D. Q. Peng, Y. S. Kim, M. H. Bae, Y. H. Jo, and H. G. Lee. 2018. Correlation between blood, physiological and behavioral parameters in beef calves under heat stress. *Asian-Australas. J. Anim. Sci*. 31:919–925. doi:10.5713/ajas.17.0545

Kim, Y. H., S. H. Warren, Q. T. Krantz, C. King, R. Jaskot, W. T. Preston, B. J. George, M. D. Hays, M. S. Landis, M. Higuchi, et al. 2018. Mutagenicity and lung toxicity of smoldering vs. flaming emissions from various biomass fuels: implications for health effects from wildland fires. *Environ. Health Perspect*. 126:017011. doi:10.1289/EHP2200

Kiser, D., W. J. Metcalf, G. Elhanan, B. Schnieder, K. Schlauch, A. Joros, C. Petersen, and J. Grzymski. 2020. Particulate matter and emergency visits for asthma: a time-series study of their association in the presence and absence of wildfire smoke in Reno, Nevada, 2013-2018. *Environ. Health*. 19:92. doi:10.1186/s12940-020-00646-2

Kollanus, V., P. Tiittanen, J. V. Niemi, and T. Lanki. 2016. Effects of long-range transported air pollution from vegetation fires on daily mortality and hospital admissions in the Helsinki metropolitan area, Finland. *Environ. Res*. 151:351–358. doi:10.1016/j.envres.2016.08.003

Kovács, L., F. L. Kézér, F. Ruff, V. Jurkovich, and O. Szenci. 2018. Heart rate, cardiac vagal tone, respiratory rate, and rectal temperature in dairy calves exposed to heat stress in a continental region. *Int. J. Biometeorol*. 62:1791–1797. doi:10.1007/s00484-018-1581-8

Künzli, N., E. Avol, J. Wu, W. J. Gauderman, E. Rappaport, J. Millstein, J. Bennion, R. McConnell, F. D. Gilliland, K. Berhane, et al. 2006. Health effects of the 2003 Southern California Wildfires on children. *Am. J. Respir. Crit. Care Med*. 174:1221–1228. doi:10.1164/rccm.200604-519OC

Kushibiki, S., K. Hodate, H. Shingu, T. Hayashi, E. Touno, M. Shinoda, and Y. Yokomizo. 2002. Alterations in lipid metabolism induced by recombinant bovine tumor necrosis factor-alpha administration to dairy heifers1,2. *J. Anim. Sci*. 80:2151–2157. doi:10.2527/2002.8082151x

Latimer, K. S. 2011. *Duncan and Prasse's veterinary laboratory medicine: clinical pathology*. West Sussex, UK: John Wiley & Sons.

Le, G. E., P. N. Breysse, A. McDermott, S. E. Eftim, A. Geyh, J. D. Berman, and F. C. Curriero. 2014. Canadian forest fires and the effects of long-range transboundary air pollution on hospitalizations among the elderly. *ISPRS Int. J. Geo-Inf.* 3:713–731. doi:10.3390/ijgi3020713

Littell, J., D. McKenzie, D. Peterson, and A. Westerling. 2009. Climate and wildfire area burned in western U.S. ecoprovinces, 1916–2003. *Ecol. Appl.* 19:1003–1021. doi:10.1890/07-1183.1

Littell, J. S., E. E. Oneil, D. McKenzie, J. A. Hicke, J. A. Lutz, R. A. Norheim, and M. M. Elsner. 2010. Forest ecosystems, disturbance, and climatic change in Washington State, USA. *Clim. Change*. 102:129–158. doi:10.1007/s10584-010-9858-

Liu, Y., E. Austin, J. Xiang, T. Gould, T. Larson, and E. Seto. 2021. Health Impact Assessment of the 2020 Washington State Wildfire Smoke Episode: excess health burden attributable to increased PM2.5 exposures and potential exposure reductions. *Geohealth*. 5:e2020GH000359. doi:10.1029/2020GH000359.

Liu, J. C., G. Pereira, S. A. Uhl, M. A. Bravo, and M. L. Bell. 2015. A systematic review of the physical health impacts from non-occupational exposure to wildfire smoke. *Environ. Res.* 136:120–132. doi:10.1016/j.envres.2014.10.015

Marlon, J. R., P. J. Bartlein, D. G. Gavin, C. J. Long, R. S. Anderson, C. E. Briles, K. J. Brown, D. Colombaroli, D. J. Hallett, M. J. Power, et al. 2012. Long-term perspective on wildfires in the western USA. *Proc. Natl. Acad. Sci. U.S.A.* 109:E535–E543. doi:10.1073/pnas.1112839109

Martin, B. L., L. C. Thompson, Y. Kim, W. Williams, S. J. Snow, M. C. Schladweiler, P. Phillips, C. King, J. Richards, N. Haykal-Coates, et al. 2018. Acute peat smoke inhalation sensitizes rats to the postprandial cardiometabolic effects of a high fat oral load. *Sci. Total Environ.* 643:378–391. doi:10.1016/j.scitotenv.2018.06.089

Mautz, W. J. 2003. Exercising animal models in inhalation toxicology: interactions with ozone and formaldehyde. *Environ. Res.* 92:14–26. doi:10.1016/s0013-9351(02)00024-5

McGuirk, S. M. 2008. Disease management of dairy calves and heifers. *Vet. Clin. North Am. Food Anim. Pract.* 24:139–153. doi:10.1016/j.cvfa.2007.10.003

Morales-Ancajima, V. C., V. Tapia, B. N. Vu, Y. Liu, D. E. Alarcón-Yaquetto, and G. F. Gonzales. 2019. Increased outdoor PM2.5 concentration is associated with moderate/severe anemia in children aged 6–59 months in Lima, Peru. *J. Environ. Public Health*. 2019:1–8. doi:10.1155/2019/6127845

Myers, R. H. 1990. *Classical and modern regression with applications*. 2nd edn. Boston, Massachusetts, USA: PWS Kent.

Nagy, R. C., E. Fusco, B. Bradley, J. T. Abatzoglou, and J. Balch. 2018. Human-related ignitions increase the number of large wildfires across U.S. ecoregions. *Fire*. 1:4. doi:10.3390/fire1010004

NASS. 2022. Cattle. USDA National Agricutural Statistics Service (NASS). https://downloads.usda.library.cornell.edu/usda-esmis/files/h702q636h/pn89f870n/jw828f69f/catl0122.pdf. Accessed January 10, 2023.

Navarro, K. M., M. T. Kleinman, C. E. Mackay, T. E. Reinhardt, J. R. Balmes, G. A. Broyles, R. D. Ottmar, L. P. Naher, and J. W. Domitrovich. 2019. Wildland firefighter smoke exposure and risk of lung cancer and cardiovascular disease mortality. *Environ. Res.* 173:462–468. doi:10.1016/j.envres.2019.03.060

NIFC. 2021. Wildland fire summary and statistics annual report. NICC National Interagency Fire Center. https://www.nifc.gov/sites/default/files/NICC/2-Predictive%20Services/Intelligence/Annual%20Reports/2021/annual_report_0.pdf. Accessed January 10, 2023.

Nikolić, M., D. Nikić, and A. Stanković. 2008. Effects of air pollution on red blood cells in children. *Pol. J. Environ. Stud.* 17:267–271. doi:10.1515/jbcpp-2013-0062.

Nowacki, G. J., and M. D. Abrams. 2008. The demise of fire and "Mesophication" of forests in the Eastern United States. *BioScience*. 58:123–138. doi:10.1641/b580207

Parks, S. A., C. Miller, M. -A. Parisien, L. M. Holsinger, S. Z. Dobrowski, and J. Abatzoglou. 2015. Wildland fire deficit and surplus in the western United States, 1984–2012. *Ecosphere*. 6:art275–art213. doi:10.1890/es15-00294.1

Ramos, C., R. Cañedo-Mondragón, C. Becerril, G. González-Ávila, A. L. Esquivel, A. L. Torres-Machorro, and M. Montaño. 2021. Short-term exposure to wood smoke increases the expression of pro-inflammatory cytokines, gelatinases, and TIMPs in Guinea pigs. *Toxics*. 9:227. doi:10.3390/toxics9090227

Reisen, F., S. M. Duran, M. Flannigan, C. Elliott, and K. Rideout. 2015. Wildfire smoke and public health risk. *Int. J. Wildland Fire*. 24:1029. doi:10.1071/wf15034

Roland, L., M. Drillich, and M. Iwersen. 2014. Hematology as a diagnostic tool in bovine medicine. *J. Vet. Diagn. Invest.* 26:592–598. doi:10.1177/1040638714546490

Roth, J., and M. Kaeberle. 1982. Effect of glucocorticoids on the bovine immune system. *J. Am. Vet. Med. Assoc.* 180:894–901.

Schreiber-Gregory, D. N., and H. M. Jackson. 2017. Multicollinearity: what is it, why should we care, and how can it be controlled? Proceedings from the Southeast SAS User Group Conference. Paper 1404-2017.

Sokolik, I. N., A. J. Soja, P. J. DeMott, and D. Winker. 2019. Progress and challenges in quantifying wildfire smoke emissions, their properties, transport, and atmospheric impacts. *J. Geophys. Res. Atmos.* 124:13005–13025. doi:10.1029/2018jd029878

Stein, A. F., R. R. Draxler, G. D. Rolph, B. J. B. Stunder, M. D. Cohen, and F. Ngan. 2015. NOAA's HYSPLIT atmospheric transport and dispersion modeling system. *Bull. Am. Meteorol. Soc.* 96:2059–2077. doi:10.1175/bams-d-14-00110.1

Tothova, C., O. Nagy, and G. Kovac. 2014. Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review. *Vet. Med.* 59:163–180. doi:10.17221/7478-vetmed

Tsai, C. Y. 2021. *The relationship between nutrient metabolism and health measures during the periparturient period in Pacific Northwest dairy herds* [*M.S. thesis*]. Animal and Veterinary Science, University of Idaho.

Uhlar, C. M., and A. S. Whitehead. 1999. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur. J. Biochem.* 265:501–523. doi:10.1046/j.1432-1327.1999.00657.x

Unosson, J., A. Blomberg, T. Sandström, A. Muala, C. Boman, R. Nyström, R. Westerholm, N. L. Mills, D. E. Newby, J. P. Langrish, et al. 2013. Exposure to wood smoke increases arterial stiffness and decreases heart rate variability in humans. *Part. Fibre Toxicol.* 10:20. doi:10.1186/1743-8977-10-20

Urbanski, S.P., W.M. Hao, and S. Baker. 2008. Chapter 4 chemical composition of wildland fire emissions. In: Bytnerowicz, A., M. J. Arbaugh, A. R. Riebau, and C. Andersen, editors. *Wildland fires and air pollution*. Elsevier.

van Leenen, K., J. Jouret, P. Demeyer, P. Vermeir, D. Leenknecht, L. Van Driessche, L. De Cremer, C. Masmeijer, F. Boyen, P. Deprez, et al. 2021. Particulate matter and airborne endotoxin concentration in calf barns and their association with lung consolidation, inflammation, and infection. *J. Dairy Sci.* 104:5932–5947. doi:10.3168/jds.2020-18981

Venn-Watson, S., C. R. Smith, E. D. Jensen, and T. Rowles. 2013. Assessing the potential health impacts of the 2003 and 2007 firestorms on bottlenose dolphins (Tursiops trucatus) in San Diego Bay. *Inhal. Toxicol.* 25:481–491. doi:10.3109/08958378.2013.804611

Vicente, A., C. Alves, A. I. Calvo, A. P. Fernandes, T. Nunes, C. Monteiro, S. M. Almeida, and C. Pio. 2013. Emission factors and detailed chemical composition of smoke particles from the 2010 wildfire season. *Atmos. Environ.* 71:295–303. doi:10.1016/j.atmosenv.2013.01.062

Wegesser, T. C., K. E. Pinkerton, and J. A. Last. 2009. California Wildfires of 2008: coarse and fine particulate matter toxicity. *Environ. Health Perspect.* 117:893–897. doi:10.1289/ehp.0800166

Westerling, A. L. 2016. Increasing western US forest wildfire activity: sensitivity to changes in the timing of spring. *Phil. Trans. R. Soc. B.* 371:20150178. doi:10.1098/rstb.2015.0178

Westerling, A. L., H. G. Hidalgo, D. R. Cayan, and T. W. Swetnam. 2006. Warming and earlier spring increase Western U.S. forest wildfire activity. *Science*. 313:940–943. doi:10.1126/science.1128834

Yang, F., W. E. Friedrichs, A. L. Navarijo-Ashbaugh, L. A. deGraffenried, B. H. Bowman, and J. J. Coalson. 1995. Cell type-specific and inflammatory-induced expression of haptoglobin gene in lung. *Lab. Invest*. 73:433–440.

Yang, F., D. J. Haile, F. G. Berger, D. C. Herbert, E. Van Beveren, and A. J. Ghio. 2003. Haptoglobin reduces lung injury associated with exposure to blood. *Am. J. Physiol. Lung Cell. Mol. Physiol*. 284:L402–L409. doi:10.1152/ajplung.00115.2002

Ye, T., R. Xu, X. Yue, G. Chen, P. Yu, M. S. Z. S. Coêlho, P. H. N. Saldiva, M. J. Abramson, Y. Guo, and S. Li. 2022. Short-term exposure to wildfire-related PM2.5 increases mortality risks and burdens in Brazil. *Nat. Commun*. 13:7651. doi:10.1038/s41467-022-35326-x