Use of Accelerometers to Characterize Behavior in Beef Cattle During Health and Subclinical Disease

by

Jenna Elizabeth Bayne

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
December 16, 2017

Keywords: bovine, remote technologies, accelerometer, sickness behavior

Approved by

Paul H. Walz, Chair, Professor of Pathobiology
Thomas Passler, Associate Professor of Clinical Sciences
James G. W. Wenzel, Professor of Clinical Sciences
Terry D. Brandebourg, Associate Professor of Animal Sciences
Brad J. White, Professor of Clinical Sciences

Abstract

The acute phase response (APR) represents a protective, coordinated response of the host with the aim of eliminating the inciting stimuli and re-establishing homeostasis. Produced by activated immune cells, proinflammatory cytokines serve as effector signals for communication between the immune system and other parts of the body, including the brain. The APR is comprised of complex neurologic, immune, endocrine, and metabolic changes. The actions of proinflammatory cytokines also elicit behavioral changes. These sickness behaviors include depression, lethargy, anorexia, and reduced activity. Detection of disease in livestock species is challenging, given the nature of these prey species to conceal signs of illness to human observers. Improvement in the health, welfare, and production of cattle has included the development and application of remote monitoring technologies. These technologies are capable of objectively quantifying physiological parameters and behaviors. One such technology used to measure sickness behavior in cattle is the three-dimensional (3-D) accelerometer, which can be applied and used to quantify lying, standing, walking behaviors, under various experimental and naturally occurring conditions.

Use of remote monitoring technologies to detect behavioral changes indicative of disease was the objective of our experimental studies. In the first experiment, the objective was to assess sickness behaviors during mild experimental endotoxemia. Lipopolysaccharide (LPS) was delivered using implanted osmotic mini-pumps (OMP) to weaned beef calves. The LPS (*E. coli* O55:B5) was delivered at 30 μg/(kg · d) for 7 days to principal calves, with an equivalent

volume of saline delivered to control calves implanted with OMPs. Use of OMP was novel for the low-dose, prolonged time course of LPS administration in cattle. Changes in traditional markers of inflammation were observed, including significant differences in rectal temperature, body weight, fibrinogen, albumin, and platelets between groups. Objective quantification of behavioral changes, using accelerometers, demonstrated significant differences between groups, with LPS calves spending more time lying, less time standing, and more time walking post-LPS infusion.

In our second study, sickness behaviors using 3-D accelerometers were evaluated in weaned beef calves following experimental infection with bovine viral diarrhea virus (BVDV). Ten weaned beef calves were inoculated intranasally with 4×10^6 $TCID_{50}$ with the low-virulent BVDV strain 134F, and control calves (n = 10) were intranasally administered BVDV-free medium. The challenge model was very mild, with mean time allotted to each activity not differing significantly between groups on any day except day 8 post-inoculation. On day 8, BVDV calves spent less time standing than controls. Following virus inoculation, calves in both groups tended to spend more time lying and less time walking and standing compared to their respective baselines. Although subtle changes in behavior were appreciable in BVDV-infected calves, the use of accelerometers could not distinguish calves subclinically infected with BVDV from healthy controls.

Use of remote monitoring technologies to demonstrate deviation from normal baseline behavior has been applied for the detection of normal physiological states, including estrus and parturition. Use of accelerometer-based activity data for the prediction of parturition has been primarily evaluated in housed dairy cattle. The aim of our third study was to characterize behavioral changes using accelerometers during the peripartum period in pastured beef cattle.

Activity data were collected from 40 multiparous and 40 primiparous mixed-breed beef cows using accelerometers, housed on pasture. Data represented two successive fall calving seasons. Within 24 hours of parturition, changes in activity indices were demonstrably different from baseline. Time spent lying and standing decreased and increased, respectively, as well as increases in lying bouts frequency and steps.

The fourth study, paired with the peripartum cow behavior study, characterized the behavior of neonatal calves during the first 7 days of life, using accelerometers. There is limited research utilizing remote monitoring technologies in young calves, especially pastured beef animals. Concurrently, measures of transfer of passive immunity and weight gain were assessed. Activity data and other parameters were collected from a total of 70 mixed-breed beef calves. During the first week of life, calves gradually spent more time standing, less time lying, and a reduction in lying bout duration. Calves born to multiparous dams had significantly higher IgG concentrations and increased weight gains compared to calves born to primiparous dams. A positive correlation between change in body weight and IgG status on day 7 was present in both primiparous and multiparous groups. Correlations between behavioral indices and IgG were found in calves of multiparous dams only. Calves with greater IgG concentrations demonstrated reduced activity.

Overall, accelerometers were successfully applied and tolerated by pastured beef cows and calves. The results of our research indicate accelerometers were useful in the quantification of behavioral changes during naturally occurring physiological changes as well as during experimental subclinical disease incidents in cattle.

Acknowledgments

I would like to express my gratitude to the members of my graduate committee, Drs. Paul Walz (chair), Thomas Passler, James Wenzel, Terry Brandebourg, and Brad White for their expertise, guidance, and assistance. I would especially like to acknowledge Dr. Thomas Passler for his mentorship, constructive criticism, and never failing support during my clinical training and graduate studies. Moreover, for his friendship during the past years and into the future. Appreciation is extended to Dr. Edzard van Santen for his statistical assistance, as well as to Drs. Manuel Chamorro and Deborah Haines for the measures of passive transfer status in neonatal calves. This work would not have been possible without the efforts of George Fincher, Steven Ledbetter and the crew at North Auburn Beef Unit, as well as those in the laboratory, including Dr. Kay Riddell, Pat Galik, and Yijing Zhang. I appreciate all of their hard work, organization, expertise, and assistance to these and ever-ongoing studies at Animal Health Research Laboratory.

I would like to express appreciation to my colleagues in the Auburn University Food Animal Section for their collegiality and inclusion in the Auburn Family. I would like to express my heartfelt gratitude for the love and support of my mother Kristi Douglas. Alike, the love and encouragement of my best friend Jocelyn Gibson, as well as that of my family, neighbors, and colleagues back home in Canada. Eternal praise to Our Lord for His immeasurable mercy and grace. Glory be to the Father, and to the Son, and to the Holy Spirit, Amen.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADG	Average daily gain
ACTH	Adrenocorticotropic hormone
AGP	Alpha-1-acid-glycoprotein
Alb	Albumin
AMS	Automatic milking systems
AI	Artificial insemination
APR	Acute phase response
APP	Acute phase proteins
ATP	Adenosine triphosphate
AUC	Area under the curve
BBB	Blood-brain barrier
BHB	Beta-hydroxybutyrate
BMP	Body movement patterns
BRD	Bovine respiratory disease
BT	Body temperature

BVDV	Bovine viral diarrhea virus
BW	Body weight
CBC	Complete blood cell count
CD	Cluster of differentiation
CI	Confidence interval
CIS	Clinical Illness Score
CMT	California mastitis test
CNS	Central nervous system
CON	Control
COX	Cyclooxygenase
CP	Crude protein
CRP	C-reactive protein
CVO	Circumventricular organs
DAMP	Danger-associated molecular pattern
DIM	Days in milk
DMI	Dry matter intake
DOF	Days on feed
EMEM	Eagle’s minimum essential medium

Fb	Fibrinogen
FBG	Fiber Bragg grating
Fe	Serum iron
FFA	Free fatty acids
GRF	Ground reaction force
Hb	Hemoglobin
HDL	High-density lipoprotein
HIS	Health index score
Hp	Haptoglobin
HPA	Hypothalamic-pituitary-adrenal
IL	Interleukin (e.g. IL-1, IL-6)
IFN	Interferon
IMU	Inertial measurement units
IP	Intraperitoneal
IRT	Infrared thermography
IV	Intravenous
KO	Knock-out
LBM	Lean body mass

LBP	Lipopolysaccharide binding protein
LDA	Left-displaced abomasum
LH	Luteinizing hormone
LPS	Lipopolysaccharide
MAP	Mitogen-activated protein kinase
Mb	Megabyte
MDBK	Madin-Darby bovine kidney
MMP	Matrix metalloprotease
MP	Multiparous
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NFκB	Nuclear factor kappaB
NPV	Negative predictive value
NRS	Numerical rating score
NVU	Neurovascular unit
Omp	Outer membrane protein
OMP	Osmotic mini-pump
PAMP	Pathogen-associated molecular pattern

PG	Prostaglandin (e.g. PGE2, PGF2α)
PI	Persistently infected
PPV	Positive predictive value
PR	Primiparous
PRR	Pattern recognition receptor
RDA	Right-displaced abomasum
RDI	Radial immunodiffusion
RFI	Residual feed intake
RFID	Radio-frequency identification
RME	Relative measure of error
ROC	Receiver operator curve
SAA	Serum amyloid A
SARA	Subacute ruminal acidosis
SC	Subcutaneous
SCC	Somatic cell count
SD	Standard deviation
SEM	Standard error of the mean
Sn	Sensitivity

Sp	Specificity
TAI	Timed artificial insemination
TCID	Tissue culture infective dose
THI	Temperature and humidity index
TLR	Toll-like receptor
TNF	Tumor necrosis factor
USST	Udder skin surface temperature
VI	Virus isolation
VLS	Visual locomotion scoring
VN	Virus neutralization
WBC	White blood cells

Chapter 1: Review of the Literature

The objectives of the literature review herein include the following. First, a description of the acute phase response (APR), including the pathways which exist between the immune system and the brain, and the sickness behaviors elicited during an APR. Second, a discussion of traditional methods used for disease detection in cattle, including subjective assessment and measurement of acute phase proteins (APP). Within the context of the APR and sickness behaviors, the third objective is a review of the current technologies available for the objective quantification of physiological parameters and behaviors in cattle.

The Acute Phase Response

The innate immune system relies primarily on pattern recognition receptors (PRRs) and associated signaling pathways, specialized chemical signaling mediators (e.g. cytokines), the complement cascade, leukocytes, and various host defense peptides.[1,2] In addition to the inherent barriers of the body, one of the first lines of defense against invading microorganisms are the phagocytic cells of the innate immune system. These include monocytes, tissue macrophages, and liver Kupffer cells.[3] These cells possess various PRRs, which include the well-characterized Toll-like receptors (TLRs).[4] PRRs bind their respective ligands, known as pathogen-associated molecular patterns (PAMPs) from invading microorganisms or endogenous danger-associated molecular patterns (DAMPs, i.e. alarmins) from damaged cells.[5,6] Lipopolysaccharide (LPS), a component of the gram-negative bacterial cell wall, is a well-known PAMP.[7] LPS binds to the

TLR-4 on monocytes / macrophages, which activates complex intracellular pathways culminating in the activation of nuclear transcription factors and synthesis of proinflammatory cytokines.[8-10] IL-1 induces its own synthesis and the synthesis of other cytokines, including those that potentiate its actions (TNFα and IL-6) and those that antagonize it (anti-inflammatory cytokines, including IL-10 and IL-1 receptor antagonists).[11-15] These inflammatory cytokines trigger acute inflammation and the acute phase response (APR), including the production of acute phase proteins (APP).[16] Furthermore, the peripheral production of proinflammatory cytokines (IL-1, IL-6, and TNFα) has significant effects on the central nervous system (CNS), including physiological and behavioral changes characteristic of sickness.[17-19] Overall, the APR encompasses the resetting of several homeostatic set points and a complex collection of neurologic, immune, endocrine, metabolic, and sickness behaviors.

Neural and Humoral Communication Pathways between the Immune System and the Brain

The concept of cytokine-induced sickness behavior has evolved over the past several decades. Kent, Bluthe, Kelley, and Danzter coined the term “sickness behavior” in 1992.[20] As reported by Besedovsky et al. (1986), administration of both purified and recombinant IL-1 to mice activated the hypothalamic-pituitary-adrenal (HPA) axis, as evidenced by an increase in plasma adrenocorticotropic hormone (ACTH) and corticosterone in IL-1 treated mice.[21] Colleagues of Besedovsky (1987) subsequently demonstrated that IL-1 directly activates corticotropin-releasing factor containing neurons in the paraventricular nucleus of the hypothalamus.[22] Subsequently, Danzter and Kelley (1989) proposed that the immune system does not work independently of the brain to regulate the host response to pathogens, and that communication is bidirectional between the immune system and brain, representing a physiological regulatory system required for host survival.[23] Cytokines represented the

molecules of communication, informing the brain as to the occurrence of infection and triggering the non-specific behavioral responses such as lethargy, depression, sleepiness, and anorexia.[17] Concurrently, Hart (1988) independently formulated cytokine-induced sickness behavior in a similar way, with the febrile response rather than the behavioral changes as a starting point.[17] The coordinated febrile response and accompanying sickness behaviors representing a state that promotes resistance to infection and facilitates recovery. By limiting metabolically taxing activities (e.g. foraging) and favoring activities that decrease heat loss and increase heat production (e.g. rest and shivering, respectively), sickness behaviors positively contribute to the host's recovery following infection.[17]

To date, an enormous amount of evidence is available on the numerous actions provoked by cytokines both centrally and peripherally, as well as the underlying mechanisms governing the communication between the periphery and the brain to elicit sickness behavior. Initiation of sickness behaviors occurs by cytokines induced by infectious agents in the periphery, as well as direct production in the brain. IL-1 and other cytokines act on the brain via two main communication pathways: a neural route and a humoral route.[24] The neural route includes the primary afferent neurons that innervate the periphery where the infectious processes occur, and relay of this sensory information to the CNS.[24] The humoral pathway involves the production of proinflammatory cytokines by immune cells in the circumventricular organs (CVOs) and choroid plexus in response to circulating PAMPs or cytokines, with subsequent propagation of this cytokine signal to other parts of the brain.[24] Several routes of immune-to-brain communication have been suggested for the action of inflammatory cytokines on the CNS, as these molecules are considered to act predominately in an autocrine or paracrine manner and are large hydrophilic peptides that cannot passively cross the blood-brain-barrier (BBB).

Vagotomy experiments in rodent models established the role of the vagus nerves in the transmission of information from the periphery to the brain. Bluthe et al. (1996) demonstrated the role of vagal afferent nerves in the transmission of an immune message from the periphery to the brain by injecting sham-operated and vagotomized rats peripherally or centrally with IL-1.[25] Vagotomy attenuated the depression in social exploration induced by intraperitoneal (IP) IL-1 administration, but did not alter the behavior-depressing effects of intracerebroventricular administration, nor did it impair immune signaling within the CNS.[25] The specificity by which afferent nerve transmission operates only within the abdominal cavity was demonstrated by Bluthe et al. (1996), in that vagotomy attenuates the decrease in social exploration induced by IP injection of IL-1 but had no effect when LPS was administered by the subcutaneous (SC) or intravenous (IV) route.[26] The decreased response of vagotomized animals to proinflammatory cytokines is not due to an inability to mount a peripheral cytokine response, as vagotomy did not alter plasma cytokine concentrations or the ability of peritoneal macrophages to produce cytokines.[26] IL-1β represents a key mediator in immune signaling from the peritoneum to the brain. As demonstrated by Goehler et al. (1999), IL-1β immunoreactivity was induced in dendritic and macrophages within the connective tissues associated with the abdominal vagus nerve within one hour after IP administration of LPS.[27] This is consistent with the idea that IL-1β is rapidly expressed by immune cells after detection of pathogens and that these cells then signal the abdominal vagus by releasing cytokines. These findings furthered the previous findings of Ek et al. (1998), which showed IL-1 receptor expression by sensory neurons of the vagus nerve, and that circulating IL-1β stimulated vagal sensory activity.[28] Marvel et al. (2004) provide further evidence for the role of vagal afferents in activating neurocircuitry which mediates sickness-induced social withdrawal behavior, through the reversible inactivation of the dorsal vagal

complex (the primary projection area of the vagus nerves) via the local anesthetic bupivacaine in rats administered LPS IP.[29] In addition, attenuated c-Fos expression (a prototypical marker of neuronal activity) was detected, supporting the immunosensory role of the vagus afferents from a neuroanatomical standpoint.[29] Other afferent nerves can be solicited when the inflammatory response takes place in different parts of the body. Injection of LPS or IL-1β into the soft palate produces a febrile response via the glossopharyngeal nerve.[30] Transection of these nerve trunks abrogated the fever response and enhanced expression of brain cytokines in response to the peripheral immune stimulus.[30]

The importance of the neural pathway in the transmission of the immune message from the periphery to the brain is not the same for all components of the APR and sickness behavior. In particular, vagal afferents were less important for the cytokine-induced fever and activation of the HPA axis than for cytokine-induced sickness behavior.[31,32] Transection of the vagus nerve inhibits fever only in response to low doses of LPS or IL-1β, whereas at higher concentrations, circulating cytokines acting on CVO override or outweigh vagal afferent input into specific areas of the brain responsible for fever generation.[26] Evidence suggests that distinct pathways of immune-to-brain communication exist and that they function in parallel with the neural pathway to bring about the different facets of the APR and sickness behavior.

The BBB is an effective barrier due to the presence of tight junctions, lack of intracellular fenestrations, and a decreased rate of pinocytosis.[33] These modifications prevent the leakage of serum proteins, including inflammatory cytokines, into the CNS. Recent evidence suggests that the function of the BBB extends beyond that of a sophisticated barrier and comprises other selective permeability, clearance, and secretory functions, a concept known as the neurovascular unit (NVU).[33] The BBB represents a critical regulatory interface between the CNS and peripheral

tissues, occupying a pivotal position in moderating humoral immunosensory communication between the CNS and periphery.[33] The neural pathway, described above, represents a fast, immediate pathway conveying peripheral inflammation to the brain and the resultant response. A slower pathway, the humoral pathway, involves the macrophage-like cells in the CVO and endothelial cells of brain vessels. Circulating peripheral PAMPs and cytokines act on these sites that lack a BBB, resulting in the local production of cytokines and molecular intermediates that diffuse into the brain. Ultimately, this results in changes in the thermoregulatory center, HPA axis activity, and onset of sickness behaviors, similar to those incited by the fast neural pathway.[24,34] A clear connection between inflammation, the immune system, and the nervous system exists, whether mediated through the neural or humoral pathways. Next, discussion of the specific molecular mechanisms underlying sickness behaviors associated with the APR are presented, including the febrile response, anorexia, depression, lethargy, and reduced activity.

Acute Phase Response and Sickness Behaviors

Fever

Fever is a well-recognized, nonspecific clinical sign of inflammation and infection. Regulation of temperature homeostasis involves the hypothalamus, which contains a central thermostat in the preoptic area that keeps core body temperature within a narrow range.[35] Fever represents a regulated increase in body temperature in response to an increased thermoregulatory set point. Differences exist in the extent certain substances can act as a pyrogen. Certain cytokines are true endogenous pyrogens, others are by-standers, and bacterial products and foreign antigens can be exogenous pyrogens (by acting on TLRs). TLRs share similar cytoplasmic signaling domains to IL-1 receptors.[4] Furthermore, IL-1β, TNF, IL-6, and other cytokines share a common pathway to activate NFκB.[4] To varying degrees, the proinflammatory cytokines IL-1β, IL-6, and TNFα can act directly on receptors in the preoptic area, effectively

reducing the firing rate of warm-sensitive neurons in the temperature-regulating region.[36] This causes the thermoregulatory set point to be stabilized at a higher point, leading to increased heat production and reduced heat loss. For example, the following chain of events may result in a fever. An exogenous or endogenous pyrogen (e.g. LPS) in the periphery or in systemic circulation induces the production of proinflammatory cytokines. Circulating cytokines subsequently diffuse into the brain side of the BBB through fenestrated capillaries and act on brain parenchymal astrocytes. These pyrogens (cytokines) act on their respective receptors (e.g. TLRs or IL-1R), resulting in nuclear NFκB activation, with resultant cyclooxygenase-2 (COX-2) and prostaglandin E synthase expression. For example, both enzymes are expressed in endothelial cells of cerebral blood vessels and perivascular macrophages following IV administration of IL-1.[37] PGE_2 diffuses into the brain parenchyma and acts on neuronal EP3 or EP4 receptors in the brainstem and hypothalamic neural structures, both of which are involved in the control of the HPA axis activity[38] and the regulation of body temperature.[39] Behaviorally, animals seek warm environments, minimize foraging activities, and assume heat-sparing postures.[17]

Anorexia

Programmed anorexia, as described by Hart (1988), may serve two potential roles: first, by not consuming food, an animal reduces the chance of raising the concentration of free iron contained within feedstuffs, which would counteract acute phase response mechanisms taking place in order to reduce the amount of free iron available to invading pathogens. [17] Second, a sick animal that does not feel hungry has little motivation to move about in search of food. Rather, it stays in one place, insulates itself from heat loss, has reduced muscle activity, and conserves energy reserves.[17] As in thermoregulation, the arcuate nucleus of the hypothalamus

plays a central role in appetite regulation. The central control of eating behavior appears to be primarily via appetite suppression, with multiple overlapping suppressing brakes resulting in anorexia.[40] Anorexia is a consistent sickness behavior demonstrable during the APR, and, like fever, appears to be mediated by proinflammatory cytokines through multiple pathways. Konsman et al. (1999) demonstrated that IP administration of LPS elicited TLR4-mediated production of IL-1β by macrophage-like cells in the CVO and choroid plexus.[41] Laye et al. (2000) showed the role of endogenous brain IL-1β in the anorexic response of IP administration of LPS in rats.[42] The observed decrease in food intake was associated with an enhanced mRNA expression of the proinflammatory cytokines IL-1β, IL-6, and TNFα in the hypothalamus.[42] Intracerebroventricular pretreatment with IL-1 receptor antagonist attenuated LPS-induced depression of foot intake and completely blocked the LPS-enhanced expression of the proinflammatory mRNA cytokine expression within the CNS.[42] This supports a role of central endogenous IL-1β in the development of the hypothalamic cytokine response, including anorexia, to systemic inflammatory stimuli. Centrally administered IL-1β into the lateral ventricle of the brain induces all the central components of the APR, including fever, HPA axis activation, and behavioral depression.[43] The IL-1β converting enzyme is responsible for the processing of inactive pro- IL-1β into mature IL-1β. Using mice deficient in this enzyme, Burgess et al. (1998) demonstrated KO mice were less sensitive to the depressing effects of LPS on food intake when injected with LPS in the lateral ventricle of the brain, whereas KO mice did not differ from controls in response to IP LPS.[44]

As described by Kim et al. (2007), the cytokines and hormones involved in anorexia following LPS injection in rats are progressive and act at different times during the process. Appetite was decreased by 2 h after a single injection of LPS and remained decreased for 24 h.

However, TNFα levels peaked before 2 h, IL-6 levels peaked between 2 and 4 h, and leptin levels peaked between 8 and 16 h. Insulin levels were increased between 2 and 16 hours, and hypothalamic cytokines were increased by 30 min after LPS injection and remained increased for 16 hours.[45] This is not to suggest that IL-1β is the sole cytokine responsible for the anorexia during an APR. Rather, a complex cytokine network exists in which a given cytokine never acts alone but rather in the context of other cytokines, either potentiating or opposing the overall activity. Likely, similar to thermoregulation, the mechanisms of cytokine-induced anorexia involve prostanoids, as COX inhibitors can ameliorate LPS- and IL-1 induced anorexia in rodents.[46,47] The reasons for why anorexia is advantageous in the face of infection remains to be fully understood. One hypothesis put forth by Bazar et al. (2005) is that filling of the intestine with food triggers a cytokine response that promotes a Th1 bias by the immune system.[48] This theory further suggests that this bias may help antiviral immunity but not antibacterial immunity. Although this theory is highly speculative, anorexia 'may' promote a Th2 response that enhances antibacterial resistance while feeding 'may' help antiviral resistance.[48] Interestingly, in human medicine there appears to be a solid link between some eating disorders and cytokine production.[49] The significance of programmed anorexia in veterinary species is not fully understood at this time, although it has been questioned if selection of animals with enhanced feed conversion in production settings selects for animals with a Th2 bias.[50]

Programmed anorexia, along with the other parts of the acute phase response, may have profound repercussions in veterinary patients with chronic diseases. Although recognized in people for thousands of years, resurgence in veterinary research efforts has occurred to explain the pathophysiological mechanisms of cachexia.[51] The weight loss that occurs in cachexia is unlike that seen in a healthy animal that loses weight due to insufficient caloric intake – in this

case, metabolic adaptations allow fat to be used as the primary fuel source, thus preserving lean body mass (LBM).[52] Conversely, acute and chronic diseases alter concentrations of a variety of mediators, including inflammatory cytokines, which decreases the body's ability to make metabolic adaptations required to switch to fat utilization, and thus amino acids continue to be used as a primary source of energy, with muscle and LBM quickly catabolized in the disease state.[52] Research efforts on the various forms of cachexia have made it increasingly clear that multiple metabolic alterations are involved in the pathophysiology of cachexia, which comprise a redundant system with multiple pathways triggering muscle loss.[52] The inflammatory cytokines, especially TNFα, IL-1β, and IL-6, are primary factors in cachexia because they cause anorexia, increase energy metabolism, and accelerate loss of LBM.[52] The mechanism of action appears to be primarily through the NFκB pathway, which has numerous effects, including increased muscle proteolysis, down-regulation of the myogenic genes myoD and myogenin, decreased muscle regeneration, and inhibition of muscle differentiation.[53,54] The catabolic effects of glucocorticoids also appear to occur by activation of NFκB, which appears to be a final common pathway in cachexia. A majority of skeletal protein catabolism in chronic inflammatory states is mediated by an ATP-ubiquitin-dependent proteasome pathway[55], but other signaling cascades can be involved.[56] This pathway involves the attachment of multiple molecules of the small peptide ubiquitin to proteins that are to be degraded, a process that requires ATP. The normal function of this pathway is to degrade any defective proteins produced in error. In the case of cachexia, the accelerated muscle degradation appears to be due, in part, to activation of this ATP-dependent pathway.[52] TNFα also affects lipid metabolism since it directly interferes with the metabolism of triglycerides and cholesterol. TNFα acts on adipose tissue cells through its receptor TNFR1 to increase both MAP kinases and c-jun.[57] This leads to activation of protein

kinase A that phosphorylates hormone-sensitive lipase and perilipins, the later regulating substrate availability for the former.[57] As a result of these activities, free fatty acids are released into the circulation, enter the liver, and provide the substrate for triglyceride synthesis.[57] Extensive research efforts continue to evaluate the role of obesity, inflammatory cytokines and leptin as significant potentiators of the immune response.[58-60] The proinflammatory cytokines of the APR, through the NFκB pathway and multiple other mechanisms, bring about anorexia during acute inflammation and significant muscle loss due to cachexia in chronic inflammatory states.

Depression, Lethargy, and Reduced Activity

Depression in animals can encompass many descriptors, including inactivity, lethargy, and listlessness or reluctance to engage with cohorts or the surrounding environment. As well, sick animals may reduce grooming behaviors.[17] Although cattle do not necessarily display the same spectrum of behavior alterations during an APR as observed in rodents, the underlying mechanisms and the primary outward depression and reduced vigor is likely similar. For example, blockade of IL-1RI with a specific antibody completely abrogated the behavioral effects of centrally and peripherally injected IL-1β in mice, whereas the blockade of IL-1RII (a decoy receptor) potentiates the suppressing effects.[61] As above, IL-1RI KO mice are no longer responsive to the behaviorally depressing effects of IL-1β injected directly into the brain or the periphery.[62] The signaling pathways that mediate the behavioral effects of IL-1 are in part associated with the NF-κB transcription factor. The central administration of a NF-κB inhibitor peptide in rabbits blocked the somnogenic and pyrogenic effects of peripherally administered IL-1β.[63] Similarly, blockade of NF-κB activation by intra-cerebroventricular administration of a cell permeant peptide that blocks the interaction of the NFKB essential modulator (NEMO) with the

IκB kinase complex abrogated sickness behavior elicited by intraperitoneal IL-1β.[64] Overall, NF-κB appears to be the major transcription factor downstream to the IL-1 signaling (and other cytokines) at the BBB (humoral) interface. Although a clearer understanding of molecular mechanisms of centrally acting cytokines is still needed, including the neurotransmitters involved within the CNS, the present understanding of sickness behavior indicates that it is the outward expression of a reversible episode of cytokine expression and action in the brain in response to peripheral immune stimulation.[24]

Proinflammatory cytokines are central to the communication pathways that link the periphery to the central nervous system. A fast acting neural and a slow-humoral communication pathway work in parallel, and ultimately converge to mediate their effects centrally to bring about complex (and incompletely understood) changes that manifest as sickness behaviors. The clinical implications of the acute phase response and sickness behaviors in livestock include the development of fever, programmed anorexia, and depression, along with altered metabolism. There exists the potential for negative consequences such as altered immune function and cachexia in prolonged, chronic disease states. Research efforts continue to characterize the molecular mechanisms that underlie the APR, as well as explaining the roles that various homeostatic alterations play in health and disease. The identification of sickness behaviors using remote monitoring technologies has the potential to improve our ability to detect disease earlier in its course in livestock, along with the expansion of our knowledge of how changes in behavior correlate with pathophysiological processes occurring in disease states. Characterization of the APR during various disease states in livestock will continue to improve with the use of these remote technologies, in conjunction with refined clinical illness scoring systems and adjunct tools, including biomarkers.

Traditional Methods of Disease Identification in Cattle

Successful identification of morbid cattle, demonstrating sickness behavior and other signs of an ongoing APR is challenging. Successful treatment of illness starts with establishing a clear case definition.[65] This case definition should be as simple yet encompassing as needed while not being cumbersome in its application. This is especially important in the livestock industry, as the daily husbandry practices, detection, and administration of treatment in sick cattle heavily relies on producers and lay personnel, all with varying levels of experience, comfort, and knowledge concerning animal behavior and pathophysiology of diseases. An example, which lends itself well to a discussion of subjective assessment and its limitations, is the identification of stocker or feedlot cattle suffering from bronchopneumonia. Bovine respiratory disease (BRD; also commonly reported as undifferentiated fever, UF) is one of the most important health problem in the feedlot industry.[66] Identification of affected cattle is heavily reliant on the detection of clinical signs by pen-checkers. At present, a perfect antemortem diagnostic test for BRD is nonexistent.[67] Clinical signs used in the identification of BRD commonly include depression, reduced feed intake or rumen fill, increased or altered respiratory effort, nasal discharge, and/or increased rectal temperature along with other secondary signs. Use of clinical illness scores (CIS) facilitate standardized evaluation of animals, with the assignment of values that correspond to the probability of a specific outcome.[68] The CIS serve to improve consistency among observers and are an adjunct tool, not a stand-alone appraisal of the animal.[68] Agreement between observers can be used to determine the repeatability (i.e. measure of precision) of the CIS. The sensitivity and specificity of a test determine the accuracy of the CIS; in other words, how well the test identifies the true disease status of an individual.[69] Semi-quantitative CIS, varying in parameters and complexity in

application have been reported in several BRD studies.[69-73] For BRD, this definition of CIS is particularly complex with respect to the varied outcomes of interest possible. This may include disease severity, lung consolidation, as well as quantifiable impacts of disease on morbidity (actual and proxy measures), mortality, production and performance parameters.

Studies detected an association between the presence of pulmonary lesions at harvest and reduced performance (e.g. average gaily gain, ADG) in feedlot cattle.[74,75] However, at present, no standard exists for defining a percentage of pulmonary involvement that would require medical intervention for cattle with BRD.[69] Additionally, knowledge of prior disease and/or treatment is limited under field conditions.[76] Nonetheless, use of lung consolidation scores as a measure to evaluate the effect of BRD is useful. For example, Thompson et al. (2006) reported an overall average reduction of 23 g for ADG when lung lesions were present at harvest. Increased severity of lung lesions resulted in greater ADG losses of 88 g.[77] Based on clinical signs and treatment records, reductions in average daily gain (ADG) of 169 g and 21 g in the early phase and overall feeding period, respectively, were found in calves treated for BRD.[77] Some have suggested ADG to be a better indicator of respiratory disease than clinical sign-based diagnosis, although not without its own confounders.[76] Using Bayesian methods, White and Renter (2009) report the use of lung consolidation scores for BRD detection to have an estimated diagnostic sensitivity and specificity of 77.4% and 89.7%, respectively.[78] In contrast, Timsit et al. (2016) report a 91% sensitivity and 67% specificity of lung scores for the diagnosis of BRD.[67] Lung consolidation scores, however useful in the post-mortem diagnosis of BRD, do not provide antemortem detection of disease.

Lung lesion scoring at harvest demonstrate the gap between CIS and accurate BRD detection, with ≥ 50% of pulmonary lesions detected at slaughter present in calves never treated

for BRD.[74,77,79,80] Diagnosis of BRD based on clinical signs and increased rectal temperature has a reported sensitivity and specificity of approximately 60%.[78] Thompson et al. (2006) found calves treated for BRD were more likely to have lung lesions than calves not treated, with a relative risk of 1.49 (95% CI 1.35 to 1.65, $P < 0.001$). However, 69.5% of animals with evidence of lung lesions at harvest were never treated for BRD. In addition, 16.9% of animals treated for BRD did not demonstrate lung lesions at harvest.[77] Amrine et al. (2013) evaluated the precision and accuracy of a CIS of BRD in young Holstein bull calves, using pulmonary consolidation scores at harvest, one day post-CIS, as the reference standard (i.e. true disease status).[69] The CIS demonstrated poor agreement, with a precision of 0.16 (95% CI, 0.10 to 0.24) and thus was of limited repeatability as a diagnostic test among observers.[69] Depending on the percentage of lung consolidation defining a diseased state, the sensitivity ranged from 81.7% (55.4 – 96.4% range) at ≥ 5% cutoff to 98.9% (93.9 to 99.8% range) at ≥ 30% cutoff.[69] Likewise, specificity ranged from 94.9% (81.3 – 97.3% range) at ≥ 5% cut-off to 80.8% (48.5 to 93.8% range) at ≥ 30% cutoff.[69] In pre-weaned dairy calves, a calf respiratory score chart was recently found to have similar sensitivity (62.4%) and specificity (74.1%) estimates as those found in studies evaluating feedlot cattle.[81] Using Bayesian methods, White and Renter (2009) report the use of clinical signs for BRD detection to have an estimated diagnostic sensitivity and specificity of 61.8% and 62.8%, respectively.[78] More recently, slightly greater estimates of sensitivity and specificity of observation for BRD diagnosis, 64.5% and 69.1%, respectively, were reported.[82] Using similar statistical methods, Timsit et al. (2016) report a 27% sensitivity and 92% specificity for BRD detection based on clinical signs.[67] Clearly, subjective assessment and the use of single or few measures to detect illness in cattle, as aptly demonstrated in BRD, is limited and significant improvement needs to be made to limit the impact of disease to the industry. Other tools for the

identification of morbid cattle are actively undergoing research. This includes the use of acute phase proteins and other biomarkers, chute-side ancillary tests (e.g. ultrasound, computerized auscultation technology), and remote technologies.[73,82-88] Use of quantitative, objective measures in conjunction with improvement of conventional methods will expectantly improve our ability to assess the health status and well-being of cattle.

Acute Phase Proteins of Cattle

During an APR, proinflammatory cytokines act on the liver and other target cells and tissues to bring about biosynthetic changes, including the profile of circulating proteins.[89] Induction of acute phase protein (APP) synthesis takes place mainly through the stimulatory actions of TNFα, IL-1, and IL-6, as well as other effector molecules, such as glucocorticoids, insulin, and growth factors.[90-92] Cytokines operate both as a cascade and as a network in stimulating and regulating APP production, such that the pattern of cytokine production reflective of the APR can differ under different types of inflammatory conditions, influencing the APP profile.[89] APP exhibit immunomodulatory and binding properties, and are an important component of the innate immune response and the overall regulation of different stages of inflammation. APP are by definition proteins that demonstrates a positive or negative change in concentration by at least 25% following an inflammatory stimulus.[93] Positive APP can be further categorized into major, moderate, and minor types based on the magnitude of concentration change and kinetic profile. Major APP have low to undetectable concentrations in the healthy, normal state, but increase over 100 to 1000-fold during the APR.[16] Major APP increase rapidly, reaching peak concentrations within 24 – 48 hours, and decrease readily back to baseline with resolution of the inciting stimulus.[16] Moderate APP are typically present in the blood of healthy animals but will increase 5- to 10-fold during an APR.[16] Peak concentrations are reached in 2 to

3 days after stimulation, with a slower decrease back to baseline compared to major APP.[16] Minor APP show an even more gradual course of increase and decrease during the APR, with minor (1.5 – 2 fold) increases above baseline levels.[16] Negative APP decrease in concentration following the onset of an APR. However, some negative APP decrease in serum concentration but are upregulated in extrahepatic tissues, such as the case of albumin, acting as a positive APP in mastitic milk.[94] Apart from albumin, use of negative APP is limited in cattle. Four major or moderate APP of cattle (and the focus of review herein) include haptoglobin (Hp), serum amyloid A (SAA), lipopolysaccharide-binding protein (LBP), and α_1-acid glycoprotein (AGP).[16] Other APP include the positive (moderate to minor): ceruloplasmin, fibrinogen (Fb), inter α trypsin inhibitor, α_2-HS glycoprotein, and α_1 anti-proteinase; as well as the negative APP albumin, paraoxonase, lipoprotein, and retinol binding protein.[95-103] Using APP as sensitive biomarkers for detection of disease in cattle are widely reported in the literature. However, the presence of APP in healthy physiological states should neither be overlooked nor misinterpreted. Changes in APP occur in healthy neonatal calves, peripartum cows, and cows at different stages of lactation.[104-113] Table 1 presents examples from the literature of the varied disease processes and APP profiles assayed. Thus, the signalment and production status of the animal should be considered during the evaluation of APP profiles. Table 2 presents reference ranges for APP described in healthy and disease states. Similarly, absolute values of APP are of value but comparison of absolute APP concentrations between different studies is challenging, given differences in study design, severity of disease models, and various analytical methods. Rather, trends and differences in the overall picture of the APP profiles during the APR should be the focus of comparisons between studies and conclusions.

Haptoglobin

Haptoglobin (Hp) is a tetrameric protein comprised of two α (~20 kDa) and two β (~35 kDa) chains linked by disulfide bonds.[114] In cattle, Hp is considered the most prominent APP released during inflammation, along with serum amyloid A (SAA). Hp exists in bovine serum as polymeric forms in association with albumin.[115] During health, Hp is nearly undetectable in sera (<0.1 g/l), with increase in serum concentrations >100-fold during inflammation.[116] Like other APP, the liver is considered the primary site of Hp production.[117,118] However, many other cells and tissues provide significant contributions. Hp is locally produced in the mammary gland, pancreas, salivary glands, forestomach, and several other tissues.[118-122] In addition, Hp is a major granule protein present within peripheral granulocytes in healthy cattle.[123] Hp binds to free hemoglobin (Hb), with each Hp molecule containing four binding sites, two for each Hb dimer.[124] Hp-Hb complexes are quickly removed from the circulation by liver Kupffer cells, limiting the damaging effects of free Hb.[125] Hp serves an anti-oxidant role of iron stabilization, reducing the oxidative damage to tissues and organs, the kidney in particular.[126] In addition, binding of Hb by Hp limits iron availability to invading bacteria.[127] The high Hb-binding affinity of Hp is capitalized on by measuring the activity of Hb peroxidase for indirect assay of Hp concentration.[128] Direct immunoassays, including enzyme linked immunosorbent assay (ELISA) and single radial immunodiffusion (RID) demonstrate higher sensitivity than Hb peroxidase activity-based assay.[129] Overall, Hp activity during the APR is anti-inflammatory.[130] Binding of Hp-Hb to CD163 of monocytes results in upregulation of anti-inflammatory mediators.[131] Direct interaction between Hp and neutrophils results in downregulation of lipoxygenase, cyclooxygenase, and lysosomal protease activities as well as inhibiting the respiratory burst.[130,132] Hp can dampen the deleterious effect of LPS by suppressing the release of TNF, IL-10,

and IL-12, providing protective effects *in vitro* and *in vivo*.[130] Matrix metalloprotease-9 (MMP-9), released as a result of neutrophil degranulation during active inflammation, can form complexes with Hp (MMP-9-Hp).[133,134] MMP-9-Hp is demonstrable in sera of cattle with acute inflammation, but its function is not fully understood.[133] Potentially, Hp serves as a scavenging mechanism, limiting the enzymatic activity and promoting clearance of the MMP-9.[134]

Serum Amyloid A

Serum amyloid A (SAA) is a major APP of several veterinary species, including cattle and other ruminants.[16] SAA belongs to a family of structurally related proteins that are constitutively expressed (SAA4) or upregulated during the APR (SAA1, SAA2, and SAA3).[135] As a major APP, plasma concentrations increase 100- to 1000-fold from baseline levels (~1.0mg/l serum, <0.3mg/l milk) during an APR.[136,137] SAA1 and SAA2 are the main circulating isoforms, produced by the liver.[138] SAA3 is the predominant inflammatory isoform, although detectable basal levels may occur in health.[119] SAA3 is produced mainly by extra-hepatic sites. In cattle, extra-hepatic sites of SAA production include the mammary gland (M-SAA3), adipose tissue, forestomach, and may other sites.[118,119,138-142] Interestingly, a study identified SAA as an adipokine, with adipose tissue being a major source of SAA.[143] Body condition score, gestation and stage of lactation affect SAA concentrations, with SAA expression in adipose tissue reaching a peak around parturition, followed by a steady decline throughout lactation, and an increase at dry-off.[139,141,144] SAA is also involved in cholesterol transport and metabolism, as SAA may become incorporated into high-density lipoproteins (HDL) with subsequent removal of cholesterol from inflammatory sites.[97,99,100] The other biological functions of SAA remain mostly unknown. SAA demonstrates immunomodulatory properties and serves as an opsonin. In humans and mice, SAA acts as a chemoattractant, influencing the migration, adhesion, and tissue

infiltration of phagocytes.[145] Expression of MMP by immune cells can be stimulated by SAA.[146] SAA can influence mucin expression and interfere with the adherence of pathogenic bacteria.[147] Furthermore, SAA can opsonize a wide range of gram-positive and gram-negative bacteria; in the later, interaction with the PAMP bacterial outer membrane protein (OmpA/OprF family protein) promotes bacterial phagocytosis by immune cells.[148,149]

Lipopolysaccharide-Binding Protein

Lipopolysaccharide-binding protein (LBP) is a 60-65 kDa glycoprotein found in circulation.[150] LBP belongs to the family of lipid transfer/LPS binding proteins (LT/LBP)[151], with the bovine LBP (bLBP) demonstrating 86 % similarity to the human form.[152,153] Physiological levels, approximately 2 μg/ml in cattle, increase several fold within 24 – 48 hours during the APR.[154,155] The liver is the primary site of LBP synthesis, but other sources include the salivary glands, forestomach, mammary gland, and many other tissues.[153,156] As its name implies, LBP binds the amphipathic lipid A moiety of LPS. Formation of LPS-LBP complexes facilitate the presentation of LPS to $CD14^+$ cells, leading to the activation of the TLR-4 and resulting inflammatory response.[157] LBP is not an absolute requirement for TLR4 activation, but greatly facilitates the interaction between LPS and $CD14^+$ by 100 to 1000-fold.[158] LBP also recognizes other PAMPs of gram-positive bacteria, resulting in the activation of other TLR pathways.[157] Thus, the immunomodulatory effect of LBP is primarily to amplify the immune response to bacteria; however, a dampening effect of LBP can be observed, likely to reduce excessive systemic inflammation.

Alpha-1-Acid-Glycoprotein

Alpha-1-acid-glycoprotein (AGP, also known as orosomucoid) is a 42-kDa heavily glycosylated glycoprotein and is a moderate, positive APP of cattle.[159] Physiological levels ranging from 0.3 to 0.5 mg/ml, depending on the species, but increasing 2 to 5-fold during an APR.[160] Structurally, AGP belongs to the lipocalin family, a ubiquitous group of proteins involved in binding, transportation, and sequestration of small hydrophobic molecules, with resultant immunomodulatory properties. AGP influences several activities of phagocytes, including modulation of apoptosis, impairment of chemotactic response, alteration of aggregation kinetics, and inhibition of superoxide production.[161-167] During an APR, AGP is able to induce or enhance the secretion of several cytokines by mononuclear cells, such as IL-1β, IL-6, and TNFα, dependent on other molecules present during inflammation.[168-170] Given its high sialic acid content, AGP may serve as an anti-infectious agent as a nonspecific competitor for cell surfaces, blocking the binding and invasion of infectious agents.[171-173] The liver primarily synthesizes AGP, but extra-hepatic synthesis occurs in the mammary gland, uterus, spleen, salivary gland, and other sites.[119,156,170,173-175]

Investigation of APP as biomarkers of disease in cattle has been ongoing for several decades. Characterization of APP profiles during inflammatory and infectious disease processes have been described for both experimental and natural occurring conditions (Table 1). These include: experimental bronchopneumonia models using viral and bacterial challenge[176-182], naturally occurring bovine respiratory disease (BRD) complex[183-187], exposure to persistently-infected BVDV cohorts[188,189], clinical and subclinical mastitis[190-195], puerperal metritis[196,197], endometritis[198-200], parasitic infections[201-205], traumatic reticuloperitonitis[206,207], abomasal displacement[208,209], sepsis[133], surgical trauma, hepatic lipidosis[210,211], and claw disorders.[212-214]

Routine husbandry practices also influence APP profiles in cattle, including ration formulation, mineral supplementation[215], vaccination[216-218], weaning, castration[219], commingling, and transportation. Use of experimental infections has improved the understanding of pathophysiology during disease, including the APP profile elicited during the APR. Utilization of APP under naturally occurring disease has focused on the economically important diseases of mastitis, metritis, and BRD, as well as nutritional management of cattle. Review of all potential disease processes and associated APP profiles is beyond the scope of this section. Rather, several studies evaluating the APR and associated APP biomarkers of BRD will be highlighted, with inclusion of other studies in the references. There is continued interest in applying APP for the differentiation of healthy from diseased, improving diagnostic accuracy, and overall assessment of health at the individual and group level under field conditions.[220-225] Equally, the use of APP as biomarkers may have potential value in the prediction of disease severity, time course of disease processes, and monitoring treatment responses.[85]

Experimental models highlight the importance of timing of APP determination during the course of disease, often a luxury not afforded under natural conditions. Single pathogen challenge studies demonstrate the ability of both viral and bacterial agents, alone, to elicit an APR (for examples, see ref[179,226,227] and Table 1). Ongoing research in human and veterinary medicine has sought to evaluate the utility of APP panels to define disease processes and potentially specific etiological agents based on unique APP profiles. Subtle differences exist in APP profile magnitude and temporal patterns in viral, bacterial, and mixed pathogen disease models. In calves sequentially challenged intranasally with bovine herpesvirus-1 (BHV-1, day 0) and *Mannheimia hemolytica* (day 4), 10% of calves had detectable Hp on day 4 whereas on day 5 43% had increased Hp concentrations.[228] By day 8, 83% of calves had increased (peak) Hp

levels.[228] Febrile responses did not parallel increases in Hp, as increased temperatures were observed 48 h after viral challenge, whereas overall clinical assessment and severity of disease coincided with bacterial pneumonia and increased Hp concentrations.[228] The APR of calves challenged with either bovine viral diarrhea virus (BVDV), *M. hemolytica* (Mh), or a combination of both demonstrated similar changes in APP magnitudes but different temporal patterns.[177] Increases in Fb and SAA were observed at (< 24h and 1-2 d) and (4-8 d and 2-4 d) post-inoculation for the Mh and BVDV challenged groups, respectively, whereas the BVDV-Mh group had a biphasic response.[177] As expected, Fb concentration increases were protracted in onset, but similar differences in temporal patterns were observed between the treatment groups.[177] The overall duration of increased concentrations of APP was significantly longer in the BVDV-Mh group compared to the BVDV, but not Mh alone.[177] Although there is some possibility in distinguishing bacterial from viral infection using APP profiles, the overlap of APP profiles precludes the use of known biomarkers for the identification of specific etiological agents. Other disease processes offer similar conclusions. Inoculation of both gram-positive and gram-negative bacteria in the mammary gland elicit increases in APP, with magnitude and duration of APP profiles reflective of mastitis severity and not underlying etiological agent.[136,229-232] As a result of increased abomasal tension and luminal pressures, increases in Hp and SAA can be detected in both left and right displaced abomasum (LDA and RDA), and thus APP profiles are not necessarily useful in distinguishing between different surgical conditions.[208,209] Nor can Hp or SAA distinguish hepatic lipidosis from abomasal displacement, conditions often occurring simultaneously in early lactation dairy cows.[208] This lack of specificity is not surprising, given the APR is a nonspecific indicator of inflammation, regardless of inciting cause. From a clinical relevance perspective, the use of APP for disease detection,

quantification of severity, and serial monitoring of progression during treatment will likely prove more beneficial.

Understood from a mechanistic, pathophysiological standpoint, BRD under naturally occurring conditions should result in similar APP changes as observed during experimental challenge models. However, translation of experimental challenge models to field conditions unfortunately is not straightforward or equal. Thus, discordant findings are reported for the diagnostic value of APP in the detection, monitoring of disease severity, and treatment response in cattle with bronchopneumonia. Difficulties in characterizing the APR and APP profiles under naturally occurring disease include correctly identifying time of disease onset, correct timing of sample collection during APR, and the multitude of other stresses that contribute to the APR. For example, Carter et al. (2002) detected a significant correlation between on-arrival Hp and SAA concentrations and the subsequent need for BRD antimicrobial treatment.[233] Furthermore, significant differences of Hp concentrations at first treatment for bronchopneumonia existed between calves requiring one compared to multiple treatments.[233] Similarly, Berry et al. (2004) detected an increased Hp concentration in calves requiring more than one antimicrobial treatment compared to those pulled once.[234] Idoate et al. (2015) demonstrated good accuracy in the use of Hp and LBP, but not transferrin (a negative APP) for the diagnosis of BRD during the first 28 d on feed (DOF).[185] Humblet et al. (2004) demonstrated positive and negative predictive values of 70% and 80%, respectively, for parallel determination of Hp and Fb concentrations in the need for antibiotic and anti-inflammatory administration at the onset of bronchopneumonia.[235] Combination of two quantitative parameters, rectal temperature and Hp concentration, demonstrated an increased discriminatory ability to identify morbidity in group-housed dairy calves compared to individual parameters alone, with a 64% sensitivity and 71% specificity

found.[225] Holland et al. (2011) report mixed results, as on-arrival Hp concentrations were not associated with overall performance (including morbidity rate), but potentially could identify calves requiring multiple antimicrobial treatments.[222] In contrast, Burciaga-Robles et al. (2009) found on-arrival Hp concentrations to have limited value for the accurate prediction of BRD with an inability for distinguishing calves requiring one versus multiple antimicrobial treatments.[184] Similarly, another study demonstrated that on-arrival Hp concentrations had a low correlation with subsequent number of antimicrobial treatments for bronchopneumonia.[71] As reported by Young et al. (1996), Hp concentrations increased in all calves with increasing number of days on feed (DOF) and observed differences in Hp concentration between healthy and BRD affected calves were small ($P < 0.1$), with poor positive predictive values.[236] Similarly, the ability to predict pulmonary lesions at harvest based on the highest Hp concentration obtained was low.[236] Calves suffering from BRD demonstrated a wide range of Hp concentrations at first pull (0 to 508 mg/dl), which was not predictive of recovery success.[237] Calves treated had a greater decrease in Hp levels compared to non-treated affected cohorts at the end of the treatment period.[237] However, Hp concentrations at the end of treatment were not predictive of lung lesions at harvest.[237] Abdallah et al. (2016) summarize the incongruity of APP value in BRD diagnosis in a recent systematic review.[238] Using meta-analytical methods, the diagnostic utility of Hp, SAA, and fibrinogen for identifying naturally occurring BRD were evaluated. Sensitivity ranges of Hp, SAA, and fibrinogen were 61 – 100%, 53 – 100%, and 57 – 80%, respectively.[238] Correspondingly, specificity ranges were 80-100%, 43-94%, and 89-95%, respectively.[238] Based on ROC analysis, Hp demonstrated the greatest accuracy of the APP evaluated.[238] However, the overall conclusion of the authors was that no firm conclusion on the diagnostic value of using APP to rule-in or rule-out BRD compared to traditional approaches was possible.[238] The

importance of bronchopneumonia as a major production limiting disease of cattle on-feed suggest there may be value for the application of APP profiles during veterinary antemortem and postmortem inspection at harvest to improve food safety and meat quality, along with many other disease conditions. Healthy cattle at harvest typically do not have significant increases in APP, allowing differentiation of healthy from diseased cattle. In bob-veal calves, Hp was not useful in distinguishing severity of lesions at harvest, but the positive and negative predictive values of an increase in Hp were 64% and 90%, respectively.[239] In emergency-harvested dairy cows, Hp and AGP concentrations were useful for differentiation of diseased animals from those with a normal clinical condition, with Hp concentrations 6-fold higher in cows with severe infectious lesions compared to those with minor lesions.[240] However, APP could not quantitatively predict meat inspection results (e.g., carcass condemnation rate or trim percentage).[240] Similar, cull dairy cows had 40-fold and 7-fold increases in Hp and SAA, respectively, compared to healthy beef type animals.[241]

Further refinement of subjective assessment methods, including the standardization of clinical illness scores and their evaluation under varied field conditions is merited. As well, continued evaluation of APP and other biomarkers is needed to improve disease detection in cattle. These traditional disease detection methods may be improved by the use of remote technologies.

Remote Monitoring Technologies

Application of remote monitoring technologies has dramatically increased in cattle production. Use of these technologies for the remote, noninvasive acquisition of objective physiological and behavioral data provides opportunities for improvement in productivity, health, and animal welfare. The objectives of the following section are to describe current

monitoring technologies used in cattle production, their application in the detection of normal physiological events, as well as the quantification of sickness behaviors in moribund cattle.

Temperature Monitoring

Veterinarians and producers regularly measure body temperature (BT) to monitor physiological and pathological processes in cattle. Cattle demonstrate diurnal variation in BT (minimum and maximum BT in the morning and evening, respectively), and a typical average daily BT within the range of 38.0 to 39.4 ºC.[242] Various external and internal factors influence BT, such as overall health, level of activity, estrus, pregnancy status, eating and drinking behavior, days in milk, milk yield, time of day, and current climatic conditions.[242-252] Since the stage of estrous cycle impacts BT in cattle, the detection of estrus has been evaluated by several different modalities, including serial evaluation of rectal, vaginal, and milk temperatures, use of intra-vaginal probes and rumen boluses, as well as infrared thermography and tail-base sensors for evaluation of skin temperatures. Similarly, changes in temperature are useful in the prediction of calving onset. Various anatomical locations have been utilized for the measurement of BT in cattle, including the rectum, ear (tympanic), vagina, reticulorumen, intraperitoneal cavity, and udder (skin, subcutaneous tissue, and milk), and agreement between the various sites has been established.[246,250,253-260] Quantification of BT is a common practice in beef and dairy standard operating protocols, although controversy exists over temperature thresholds consistent with disease and the need for intervention.[261]

Transition dairy cow management includes scrutiny of early postpartum cows for the early recognition of sick cows by farm personnel. During the first 14 DIM, various parameters (e.g. BT, attitude, milk production, uterine discharge, ketones) are evaluated for the identification of common peripartum diseases.[262] Daily rectal temperature evaluation can detect changes 72 to

48h before the diagnosis of puerperal metritis.[263] For cattle on feed, traditional methods (e.g. pen checking) are highly reliant on visual appraisal of animals for signs of depression, nasal or ocular discharge, lethargy, changes in respiratory effort, reduced feed intake and gut fill, or any combination for the detection of sick animals. Symptomatic animals with rectal temperatures >40.0 ºC are usually considered morbid (case definition of BRD).[264] Diagnosis of BRD based on clinical illness scores is very limited in its sensitivity[67], and is highly dependent on observer skill and disposition of cattle, as discussed previously. In calves with BRD, the febrile response severity is closely associated with worsening of clinical signs.[265] Avra et al. (2017) detected significant differences in rectal temperatures at the time of treatment and the subsequent risk of treatment failure for low-risk cattle.[266] In contrast, another study reported that rectal temperature was not predictive of the ability to finish the production cycle.[267] Although highly repeatable, rectal temperature used as a single diagnostic criterion for the presence of disease is limited.[261,268] Use of a single time-point rectal temperature is prone to type I error (i.e., fever when the animal is healthy) and type II error (i.e., no fever when the animal is sick).[261] Although frequent measurement of BT using hand held thermometers is simple, common, and relatively cost effective, it is also labor intensive, time consuming, and handling of the animal can cause increases in temperature due to stress and excitement. Recognition of the need and development of devices capable of continuously measuring body temperature in cattle is not new.[269,270] Devices capable of continuous temperature measurement and relay of data by radiotelemetry have been used primarily in research, for the detection of health events under controlled settings in real-time. Wide acceptance and use in commercial livestock production has been limited. Dependent on the technology used, this may be due to multiple factors including invasiveness of implanting device, sensor stability and retention, battery life, and the cost of equipment. With

advancement in technology capabilities and reduction in manufacturing costs, there is a renewed interest in remote temperature monitoring modalities in research and commercial production. Temperature monitoring modalities include sensors affixed or internally administered to the animal as well as remote acquisition of external temperature by infrared thermography.

Implanted Thermometry

Use of continuous thermometry using implantable temperature-sensing microchips has been evaluated in several domestic, exotic, and wildlife species.[271-276] There is close agreement between rectal temperatures and the detection of both normal and abnormal (febrile) temperature ranges by the implanted transponders. Description of products used in cattle are limited to research settings. Goodwin et al. (1998) compared tympanic infrared thermometry, subcutaneously implanted microchip thermistor, and rectal thermometry in horses, sheep, and goats.[274] Subcutaneous microchips demonstrated the greatest variability of the three methods. In goats, there were moderate to strong correlations between the three modalities, with only moderate correlations in sheep and poor correlations in horses.[274] With respect to the different modalities, temperatures increased between tympanic, rectal, and subcutaneous measurement for goats, whereas in sheep the order was rectal, tympanic, and subcutaneous.[274] Lee et al. (2015) reported the long-term evaluation of subcutaneous temperature monitors under fluctuating environmental and seasonal conditions using a subcutaneously implanted RFID thermo-logger (iButton DS1922L, Maxim Integrated, San Jose, CA)[b] in Holstein steers. Thermo-loggers accurately captured circadian temperature rhythms within a stable temperature range, over several seasons.[277] However, a significant impact of sub-zero ambient temperature on device measurement (< 37 ºC) was observed, and the authors advise that the device not be used for quantification of hypothermic states.[277] Recently, Grissett et al. (2014) utilized the Biothermal

RFID Chip (Destron Technologies, Round Rock, TX)[a] for the assessment of an intranasal modified-live virus vaccine and the impact of different ambient temperatures in cattle.[278] Commercially marketed as LifeChip® by Destron Fearing™ the device is for the permanent microchip identification of companion livestock, along with its ability to monitor subcutaneous body temperature.[a] Reports for the validation of thermometry microchips are available for dogs, horses, sheep, and goats.[274,279,280] Using a thermistor-tipped pulmonary artery catheter as the reference of core BT, Greer et al. (2007) found similar agreement between the rectal thermometer and subcutaneously implanted thermistor microchip, both of which were more reliable than auricular thermometry.[279] Although reasonable agreement between core BT and microchip-based subcutaneous temperatures were present, a difference as great as 3.5 ºC was observed, which brings into question the thermistor microchip accuracy.[279] In horses, thermistor microchips are strongly impacted by ambient temperatures and often under- or over-estimated body temperature, dependent on the current weather conditions. Based on the findings of Robinson et al. (2008), if one relied on thermistor measurement alone for the detection of fever, > 50% would be missed in cooler ambient temperatures, whereas <15% would be missed when the ambient temperature is > 15.6 ºC.[280] Although effective in monitoring temperature continuously, conveniently without the need for restraint, and relatively accurately, the use of subcutaneously implanted thermometry transponders is expected to remain limited to research settings, because the use of such devices in livestock destined for slaughter would constitute adulteration of the carcass.

Based on similar thermistor technology used in the subcutaneous implants, wearable sensors in contact with the skin for monitoring of temperature in calves and adult cattle have been developed.[281] In a pilot study by Nogami et al. (2013), the tail-base skin temperature in a

young calf was continuously monitored for 16 days to compare the daily maximum temperature to the daily maximum rectal temperature.[281] The sensor was adhered to the tail with elastic adhesive tape, had a reported accuracy of ± 3 ºC, and had activity monitoring (accelerometer) capabilities.[281] Skin temperature was 2-3 ºC lower than rectal temperature, but was in agreement with circadian rhythm increases and decreases of temperature within 24-h periods.[281] Correlation between rectal temperature and sensor skin temperature in this one calf was r = 0.8.[281] Erroneous readings were periodically observed and were likely due to loss of sensor contact with the skin.[281] Using the same skin thermistor sensor, Miura et al. (2017) monitored skin temperature throughout a complete estrous cycle in cows and heifers, during three consecutive seasons.[282] The largest change in skin temperature from baseline was on the day prior to ovulation.[282] A maximum amplitude of 0.92 ± 0.35 ºC at 21.5 - 29.5 h before ovulation, coincided with the highest LH concentration during the preovulatory spike.[282] Commercialization of such skin thermistor sensors for cattle will likely be realized in the future. However, refinement of a prototype with easier application and retention of the skin thermistor to the base of the tail is needed.

Intra-Vaginal Thermometry

The hormonal profiles and temperature changes throughout the estrous cycle in cattle have been extensively characterized, and the impact of pharmacological protocols to induce estrus on BT have been quantified using several thermometry methods.[242,283-288] Changes in milk temperature as an indirect measure of body temperature have also been evaluated using in-line sensors.[289-291] Monitoring of temperature changes can be utilized for the detection of onset of estrus for natural and artificial insemination (AI) purposes. Serial evaluation of rectal and vaginal temperatures by hand held meters is labor intensive and insensitive to cyclical temperature

changes. Development of microprocessor controlled data loggers, attached to a modified controlled internal drug release (CIDR) for retention, allow measurement of vaginal temperature using radiotelemetry. In brief, during the preovulatory period, a decline in serum progesterone (< 0.5 ng/ml) with preovulatory rises in estradiol and LH (20 to 56 hours post P4 decline) are observed, followed shortly by a temperature spike ≥ 0.3 ºC, ranging from 3 – 9 hours in duration.[285] Different magnitudes of temperature change, 0.3 to 1.3 ºC have been observed. [242,248,283,284] Longer durations in temperature spike were observed in heifers (4 – 21 hours).[286] Ovulation follows the preovulatory LH surge by approximately 24 hours (16 – 33 h).[286] The preovulatory LH peak precedes the temperature spike, ranging from 8 to 20 h; whereas estradiol peaks before, concurrent with, or after the temperature spike.[285,286] Mean duration between temperature spike onset and ovulation was 21.14 h (s.d. 6.07, n = 7) in heifers, based on laparoscopic confirmation of ovulation.[286] The interval between the onset of the temperature spike to the time of ovulation was as consistent as the intervals between the other measured hormonal profiles associated with ovulation.[286] In agreement, strong correlations ($r = 0.82$, $P < 0.05$) between ovulation with onset of standing estrus, LH peak ($r = 0.81$, $p < 0.05$), and increase in vaginal temperature ($r = 0.74$, $P < 0.05$) were reported by Rajamahendran et al. (1989).[287] Suthar et al. (2011) report the impact of PGF2α-induced estrus on body temperature using the vaginal temperature logger (Minilog8, Vemco Ltd., Halifax, Canada) attached to a modified CIDR. [248] High body temperatures (39.0 ± 0.5 ºC), with a maximum absolute increase of 0.5ºC, and low progesterone concentrations (< 0.5 ng/mL) were observed during estrus, whereas low body temperatures were observed from PGF2α injection to estrus (38.6 ± 0.3 ºC) and around ovulation (38.5± 0.2 ºC), respectively.[248]

Despite the widespread use of vaginal temperature loggers, validation of data captured by vaginal temperature loggers was performed by Vickers et al. (2010) recently and demonstrated that vaginal temperature loggers provide a reasonable measure of body temperature.[260] However, the strength of the relationship between vaginal and rectal temperature is dependent on the temperature range and the time elapsed between measures. Correlation coefficients between vaginal and rectal measures were 0.81 and 0.46 for postpartum and peak-lactation cows, respectively ($P < 0.001$).[260] In healthy cows and those with retained placenta, vaginal temperature loggers captured the diurnal rhythm of temperature changes accurately.[260]

Continuous vaginal temperature monitoring throughout the entire estrous cycle in lactating beef cows via radiometric transmitters was reported by Kyle et al. (1998).[283] An estrus-related peak in vaginal temperature was defined as an increase of 0.4ºC for 3 or more consecutive hours compared to baseline temperatures defined over 2- or 3-day periods.[283] A mean maximum in vaginal temperature of 0.9 ± 0.3 ºC at estrus was observed, with a duration of 6.5 ± 2.7 hours.[283] Vaginal temperature for 48-hours preceding the estrus rise in temperature was significantly lower than that of the previous 96-hours in 84% of cows ($P < 0.05$).[283] Use of the peak in vaginal temperature for the prediction of estrus had a sensitivity of 89.4% and a positive predictive value of 85.7%, while visual observation had a sensitivity and positive predictive value of 53.2 and 96.2%, respectively.[283] This was an improvement in estrus detection compared to the findings of Redden et al. (1993), wherein estrous was detected in 81% of cows, with an overall accuracy of 69% using vaginal temperature changes.[284] Vaginal temperature changes were reportedly more predictive of estrus compared to pedometer-based data; and, unlike locomotor activity, vaginal temperature change was not impacted by seasonal differences.[292]

In a small pilot study by Aoki et al. (2005), continuous vaginal temperature monitoring, via the use of a thermocouple sensor, was predictive of calving within 36 to 60 hours once a decrease in temperature ≥0.3 ºC was observed, with an accuracy of 74%.[293] The device described was comprised of a harness, data-logging apparatus, and a thermocouple sensor inserted into the vagina and bonded to the vulva using an adhesive.[293] This sensor configuration would be too cumbersome for commercial use, necessitating the development of more practical technologies

Other sensor types for the detection of BT changes have also been developed, whether it be for health monitoring or estrus detection. In a pilot study by Fisher et al. (2008), a prototype toroidal conductivity sensor was tested for its suitability to measure vaginal temperature and conductivity for timed artificial insemination (AI) purposes in non-lactating cows.[288] Although conductivity of vaginal mucus rose steadily until the LH surge, overall conductivity was not reliable for prediction of the LH peak due to large variability in the recorded data.[288] However, the captured temperature data was useful for prediction of the LH peak, with a mean increase in vaginal temperature at estrus of 0.48 ºC.[288]

Ear-tag Thermometry

Another temperature monitoring modality is a thermistor affixed to an RFID-ear tag, with several commercially available systems. These devices measure external ear canal temperature based on tympanic and/or external auditory canal thermometry as a proxy to core body temperature. Similar to subcutaneously implanted thermistor technology, critical assessment of this remote-monitoring modality appears to be limited in cattle, under both research and commercial settings. The FeverTag® (Fever Tags®, Amarillo, TX)[c] continuously monitors temperature and alerts the producer by a flashing LED light when ear canal temperatures exceed >39.8 ºC for 6 h. McCorkell et al. (2014) found consistent activation of the tag to be at a

temperature considerably higher than the factory-set threshold of 39.8 ºC.[294] Evaluation of auction market-derived calves for the diagnosis of BRD, based on CIS, increased rectal temperature, and hematological changes, found positive (flashing light) ear tag responses in 85% of CIS positives in an initial group of calves, whereas only 15% of CIS positives in a second group.[294] Although most ear tags flashed at the time of clinical illness detection in the first group of calves, the authors speculate discordant findings in the second group of calves were due to placement of the tag too lateral in the pinna.[294] As reported by the same research group, under experimental BVDV infection, detection of illness based on FeverTag® response was found in only 50% of calves demonstrating clinical signs of illness.[294] Although false positives were very rare, the presence of false negatives and inferiority to subjective clinical assessment warrants improvement in tag accuracy and reliability for the tags to be useful in the early detection of BRD, under commercial feedlot conditions. As reported by Mahendran et al. (2017), identification of enzootic pneumonia in housed dairy calves using the FeverTag® system is sensitive for the detection of several fever-inducing calf-hood diseases.[295] The continuous monitoring of temperature post-treatment also was beneficial for the identification of calves failing to respond to initial treatment.[295]

Rumen Thermometry Boluses

Intraruminal sensors are a noninvasive alternative to surgically implanted devices. Commercially available rumen boluses can acquire data on rumen pH and temperature, as well as provide a means of electronic identification when incorporated into the device. Examples include boluses commercially available from smaXtec (Graz, Austria)[d] and eCow (Exeter, England)[e]. With respect to safety, Antonini et al. (2006) evaluated the biological impact of rumen boluses *in vivo*, including the mechanical action of the bolus on ruminal mucosa,

alterations in reticulorumen motility, and overall animal health and performance over a two-year period.[296] Retention of the transponders was 97.6%, lower compared to previous reports ranging from 98.8% to 99.7% in various ruminants.[297-299] Over the 2-year study period, the presence of the transponder did not affect annual milk yield, milk fat yield, and milk protein yield ($P > 0.05$) or weight gain in treated cattle.[296] Similarly, treatment did not influence the reproductive traits, except conception rate, which was greater in treated cows ($P < 0.05$).[296] Treated bulls, heifers, and cows showed a lower number of chewing movements ($P < 0.01$), and treated animals tended to have a greater frequency of regurgitation ($P = 0.07$; $P = 0.26$; $P = 0.08$ bulls, heifers, and cows, respectively) when compared to controls.[296] The later finding is the first report of a change in rumination behavior due to the presence of the bolus. However, its relevance and repeatability remains to be further explored. The presence of ruminal transponders did not affect performance or reproductive traits, suggesting that modification of ruminal motility patterns was not able to exert negative effects on digestive and reproductive physiology.[296] Evaluation of postmortem localization of transponders found gross lesions within the reticulum mucosa of 9/43 animals (transponder in place for a period of 461 ± 27 days), histologically characterized by mild dystrophy and flattening of folds and papillae over a limited area.[296] Due to the activity of heat-producing rumen microorganisms within the forestomaches, reticulorumen temperature is approximately 0.5 ºC greater than rectal temperature.[255] Using rumen temperature boluses, strong correlations between rectal and reticulorumen temperatures were demonstrated indicating that reticulorumen temperature is an effective measure of core body temperature.[255] Bewley et al. (2008) demonstrated a strong correlation ($r = 0.645$, $P < 0.0001$) between reticulorumen and rectal temperatures (n >2000 observations) over a 4-year period in a commercial milking herd, under various environmental conditions. [255] Factors found to impact rectal and reticulorumen

temperatures included: season, milking, housing system, and parity.[255]Water intake, dependent on its temperature, can also lead to large fluctuations in reticulorumen temperature lasting several hours.[247] Thus, reticulorumen boluses provide a safe, noninvasive, and effective means of continuously quantifying BT and represent a useful remote monitoring tool for determination of health status in cattle.

Monitoring of reticulorumen temperature has been successfully used for the detection and prediction of disease onset in beef and dairy cattle. Rose-Dye et al. (2011) demonstrated the ability of radiotelemetric boluses to detect reticulorumen temperature increases under experimental challenge with exposure to BVDV persistently infected animals, intratracheal inoculation with *Mannheimia hemolytica,* and the combination of viral exposure and bacterial challenge in steers.[300] Rumen temperature boluses detected an immediate response to *M. hemolytica* challenge, whereas exposure to PI BVDV animals caused cyclical temperature increased during and after exposure.[300] Response to *M. hemolytica* challenge increased daily average and maximum ruminal temperatures by approximately 1.2 ºC compared to control calves ($P < 0.01$), with rumen temperatures returning to those of controls by 24 hours following bacterial inoculation.[300] Following BVDV exposure, maximum temperature increases were 0.8 ºC greater, and those for the viral and bacterial combined challenge were 1.3 ºC greater than controls ($P < 0.01$).[300] Collectively, these findings show detectable changes in reticulorumen temperature following challenge with *M. hemolytica* and BVDV exposure[300], similar to those of Burciaga-Robles et al. (2010) demonstrating changes in rectal temperature under very similar experimental conditions.[188]

Evaluating fattening bulls in France, Timsit et al. (2011) detected BRD in young bulls before the onset of clinical signs of the disease using radiotelemetric reticulorumen boluses.[301,302]

In their study, 87.5% of bulls were diagnosed with BRD. Use of rumen boluses to detect rumen hyperthermic (RH) episodes improved BRD detection by 17% over visual appraisal.[301] Furthermore, RH episodes preceded clinical disease detection, on average, by 51 hours.[301] Most bulls had 1 to 5 RH episodes, with multiple episodes separated by intervals of 3 – 19 days.[301] The mean duration of RH episodes was 60 hours (± SD 43h, range 9 – 190h), with reticulorumen temperatures ranging from 39.4 to 42.4ºC (mean ± SD, 40.6 ± 0.4ºC), and averaging 0.57 ºC ± 0.27 ºC higher than rectal temperatures.[301] Increased haptoglobin concentrations were observed during RH episodes, with concentrations > 0.13 g/L being reached in 73% of RH episodes.[301] The positive predictive value of RH episodes (>6h) for the detection of BRD cases was 73%.[301] However, the prevalence of BRD was particularly high in this study and further research to determine the utility of RH episodes in predicting BRD in groups of animals with a lower prevalence of BRD is needed. The remaining RH episodes (27%) were not predictive for BRD (14/52). Timsit et al. propose agonistic interactions (e.g. young bulls fighting), fever episodes following vaccination performed at entry, or diseases other than BRD might account for non-diagnostic RH.[301]

In a prospective case-control study by Adams et al. (2013), the feasibility of using rumen temperature boluses to aid in early disease detection in dairy cattle was evaluated.[250] Reticulorumen temperature data were captured thrice daily by a plate reader that powered the bolus, as cows exited the parlor. Significant temperature increases from baseline were predictive of pneumonia and mastitis, but not for lameness or metritis.[250] In addition, cows that developed mastitis or pneumonia had increases in RT within a 7-day period, preceding the detection of clinical signs by farm personnel.[250]

Development of rumen boluses capable of quantifying and relaying pH for research purposes negates the need for surgical implantation of pH meters.[303] Monitoring rumen pH in conjunction with temperature by incorporating both modalities into a single bolus is advantageous and provides complementary data.[304] This is important from a rumen health standpoint, for example, for the detection of subacute ruminal acidosis (SARA), a common disorder of high production dairy cattle and cattle on feed for harvest purposes. It is possible to predict rumen pH based on rumen temperature.[256,305] However, others have cautioned use of rumen temperature alone to predict pH changes, as both rumen temperature and pH are subject to circadian changes associated with fermentation.[304] AlZahal et al. (2011) evaluated the ability of radiotelemetric boluses to differentiate between rumen temperature changes that were due to subacute ruminal acidosis (SARA) or LPS induced endotoxemia.[306] In that study, feeding regimens [rations included: moderate forage: concentrate ratios (MFC) or high forage: concentrate ratios (HFC)] to generate rumen pH changes characteristic of SARA and the intramammary infusion of 100μg of LPS (*E.coli* O111:B4) to induce a systemic endotoxemia febrile response were used to compare outcome parameters.[306] The group had previously demonstrated ruminal temperature was negatively correlated with ruminal pH under SARA conditions.[256] Radiotelemetric boluses recorded a longer duration of ruminal temperature above 38.8 ºC in MFC cows compared to HFC cows, resulting in the suggestion that a threshold of 38.8ºC be used for SARA detection in nonfebrile animals.[306] Ruminal temperature during LPS-induced endotoxemia peaked on average between 40.5 to 41.0 ºC and remained above 40.0 ºC for approximately 2 hours, whereas the average daily maximum due to the dietary effect remained below 39.5 ºC.[306] Although the telemetric boluses were able to capture febrile

responses during endotoxemia, a dietary effect was not demonstrated and detection of SARA was limited to nonfebrile animals.[306]

In addition to the detection of disease, use of rumen temperature boluses enables the detection of changes in body temperature associated with the normal physiological events of estrus and parturition. Use of rumen temperature boluses in Angus beef cows by Cooper-Prado et al. (2011) demonstrated a significant decrease in rumen temperature 48 to 24 hours before the onset of parturition ($P < 0.001$), as well as a significant increase in rumen temperature at 0 to 8 hours after the detection of estrus ($P < 0.001$).[307]

Infrared Thermography

Infrared thermography (IRT) represents a non-invasive modality for the determination of peripheral skin temperature in cattle remotely and in real-time. Based on the principal that all objects emit infrared radiation proportional to their temperature via conduction, convection, and radiation according to the Stefan-Boltzmann law, a thermal camera absorbs infrared radiation and generates pictorial images based on the amount of heat generated.[308] Thermal imaging is useful for the detection of physiological and pathological changes in skin temperature associated with disease, welfare issues (e.g. heat stress and transportation), as well as the impact of routine husbandry practices (e.g. branding) on livestock.[309-317] Skin surface temperatures reflect the underlying circulation and tissue metabolism, which are subject to autonomic nervous system control. The presence of inflammation – classic signs of rubor, calor, dolor, tumor, and functio laesa – can influence surface temperature. This may allow the detection of subtle temperature changes associated with local or systemic inflammation, for example, changes in udder skin surface temperature (USST) associated with mastitis. Measurement of peripheral skin temperatures in cattle and sheep using IRT demonstrated a strong correlation with core body

temperature.[318] Byrne et al. (2017) report a high level of precision of a handheld IRT camera for surface temperature measurement when the average of three replicate images were obtained for a specific anatomical location (e.g. the eye, udder, or hooves).[319] Further, maximum temperature of the eye and the udder had the lowest error variance.[319] Experimental IRT measurements of various anatomical locations of subject animals have been reported. Hoffmann et al. (2013) report significant differences in IRT measures for different body regions: eye, ear, shoulder, and vulva.[320] Precision of IRT head and body measures were equal for adult cattle, whereas body areas compared to the head were best suited in calves in the study.[320] Whole-body IRT mapping of Jersey heifers reported by Salles et al. (2016) demonstrated a strong association between temperature and humidity index (THI) and head and flank regions.[321] Further, head regions had the highest correlations with rectal temperature.[321] Importantly, skin temperature is affected by various internal and external factors. Animal factors include coat color, density of coat, parity, stage of lactation, pregnancy status, estrus, and recent strenuous activity, which all can influence skin temperature.[322] Standardization for site preparation (e.g. cleaning, grooming), acclimation time to changes in settings, and distances for image capture have not been established. Environmental conditions reported by Church et al. (2014) which impacted IRT eye temperatures included wind speed and direct sunlight.[323]

Early work by Hurnick et al. (1985) evaluated 27 Holstein cows using IRT to characterize temperature changes associated with estrus.[324] Measurement of perineal skin temperature was performed once daily for 90 days postpartum.[324] Timing of estrus was based on serial milk progesterone measurements, with ovulation assumed at 5 days before the first sustained increase in progesterone and the day of estrus 1 day prior.[324] A rise in perineal IRT temperature associated with estrus was defined as an increase of 25% compared to the mean of

the preceding 4 days.[324] Although IRT measures increased on the day of estrus, the authors felt the technique was limited by the level of false positives (33%) and false negatives (7%).[324] Recently, Talukder et al. (2014) evaluated several thresholds of IRT vulvar and muzzle temperature change for estrus detection (IRT estrus) compared to human observation and a tail-head mounting detector.[325] IRT measurements were performed twice daily. The maximum vulvar and muzzle temperatures were observed at 24 and 72 hours, respectively, before ovulation; whereas the lowest temperatures were observed at 48 hours before ovulation for both sites. Ovulation occurred 24 to 47 hours after the onset of the IRT estrus alert in 73% of cows, with 45% occurring within 12 to 23 hours after the end of IRT estrus.[325] Overall, IRT had a greater sensitivity compared to visual observation (67%) and Estrotect activation (67%), but had a lower specificity and positive predictive value.[325] Based on different thresholds of temperature change (1, 1.25, and 1.5 °C), IRT estrus sensitivity and specificity ranged from 75 – 92% and 29 – 57%, respectively.[325] The same research group has evaluated measurement of additional anatomical sites – eye, ear, muzzle, and vulva for IRT estrus detection in pastured dairy cattle. Irrespective of anatomic site for IRT measurement, the accuracy of estrus detection was poor compared to other activity monitors (accelerometers and rumination activity) and visual observation.[326]

In the dairy sector, the use of IRT as a tool in the detection of mastitis is important for animal health and welfare. In terms of financial feasibility, the ability to detect clinical and subclinical cases of mastitis will be important for future technology development, acceptance, and implementation of IRT monitoring under commercial settings. Hovinen et al. (2008) evaluated IRT using a model of intramammary LPS-induced mastitis.[327] Increases in USST by 1-1.5ºC were associated with signs of clinical mastitis and corresponding increase in rectal temperature.[327] There was a strong correlation between maximum USST and rectal temperature

(r = 0.98, $P < 0.001$).[327] Rectal temperatures increased by 4-8 h post-infusion and peaked at 6 h, whereas local signs of inflammation within the gland and altered milk appearance were present at 2 – 4 h post-infusion.[327] Potentially due to the severity of the challenge model, increases in USST paralleled rather than preceded changes in rectal temperature, even though local signs of inflammation were appreciable by 2 h post-infusion in most cows.[327] Pezeshki et al. (2011) demonstrated IRT detection of 2-3 ºC USST changes in mastitic quarters experimentally infused with *E.coli.*[328] However, unlike the changes in temperature related to disease progression reported by Hovinen et al.[327], the peak in USST (12 h post-infusion) occurred after the appearance of clinical signs and peak rectal temperature (9 h post-infusion).[328] Using an *E. coli* intramammary infusion model in primiparous Holstein cows, Metzner et al. (2014) compared different algorithms for the serial thermographic evaluation of *E.coli*-induced mastitis.[329] Algorithms were produced using several geometric analysis tools (polygons, rectangles, and lines) and descriptive parameters (minimum, maximum, range, mean, and standard deviation). A strong correlation between udder surface temperature and rectal temperature was found for both hind quarters.[329] Under these conditions, an algorithm that used the polygon image and maximum temperature was best suited for detection of acute mastitis, detecting a 2.06 ºC increase in temperature between affected and control quarters.[329] Under experimental conditions, serial evaluation of maximum USST every 2 h was ideal for the detection of mastitis onset, as subsequent edema formation reduced the temperature variation and average USST.[330] However, frequent IRT imaging (every 2 h) would be impractical under field conditions, where IRT data capture would best be suited to coincide with milkings in a parlor or robot.

Findings of Colak et al. (2008) on the detection of subclinical mastitis by IRT analyses demonstrated a linear relationship between increasing CMT score and USST.[331] A strong

correlation between quarter temperature and CMT score (r = 0.93, $P < 0.0001$), but a weak correlation between rectal temperature and CMT score (r = 0.27, $P < 0.01$) were found.[331] Polat et al. (2010) evaluated the associations between udder skin surface temperature (USST), somatic cell counts (SCC), and California Mastitis Test (CMT) for the detection of subclinical mastitis in Brown Swiss dairy cows.[332] USST was positively correlated with SCC (r = 0.73) and CMT score (r = 0.83).[332] A linear increase in USST with an increase in CMT score was found. [332] Use of IRT for determination of USST allowed differentiation between subclinical mastitis and normal udders (based on SCC >400,000 cells/mL), with USST of subclinical mastitic quarters 2.35 ºC greater than healthy quarters.[332] Based on ROC curve analyses, sensitivity and specificity of IRT (95.6 and 93.6%, respectively) compared to CMT (88.9 and 98.9%, respectively) were not significantly different.[332] Using IRT to detect subclinical mastitis on four commercial dairy farms, Bortolami et al. (2015) included bacteriological findings in addition to SCC and USST.[333] Similar to experimental results previously, USST and SCC were significantly correlated ($P < 0.05$); however, IRT discrimination of bacteriological culture negative and positive cows was not possible.[333] Thus, IRT analysis of udder skin temperature may be sensitive enough to detect subtle temperature changes associated with both clinical and subclinical mastitis.

Use of thermography as a tool for the identification of animals suffering from BRD is growing as an area of interest in the feeder cattle sector, as hand held or mounted IRT cameras allow non-invasive assessment of animals in large group settings. Research by Schaefer et al. demonstrated the diagnostic potential of remote, real-time IRT assessment of orbital maximum temperatures for the early identification of experimental BVDV[334], as well as BRD under experimental and naturally occurring conditions.[335,336] Assessment of IRT of various anatomical locations in an experimental BVDV challenge model demonstrated significant IRT changes

predictive of disease onset.[334] Changes in IRT were detectable up to 7 d earlier compared to biological measurements, which included clinical signs and increased concentration of Hp.[334] Changes in IRT temperatures were extremely sensitive, with surface temperature changes <1 ºC found to be clinically significant.[334] Changes in maximal orbital temperatures were the most sensitive, observed as early as 1-d post BVDV-inoculation, whereas changes in nose, ear, or dorsum skin did not appear until d 5 or 6 post-inoculation.[334] In a group of commercially sourced beef calves, Schaefer et al. (2007) found IRT changes to be equal or superior for the identification of BRD compared to clinical score, core temperature, or hematological changes.[336] BRD-affected calves demonstrated higher peak IRT values compared to negative calves ($P <$ 0.01).[336] Of importance, IRT was able to detect the onset of clinical BRD by 4-6 d compared to other detection methods evaluated.[336] Improving on the application of IRT, Schaefer et al. (2012) devised a mounting bracket and automation of the IRT camera, linked with RFID tags and use of a watering station.[335] Efficient detection of BRD in a group of calves with a low prevalence of disease was demonstrable by IRT maximum orbital temperature changes.[335] In contrast, Fraser et al. (2014) found poor detection of IRT orbital temperature changes associated with experimental *Mycoplasma bovis* inoculation and the progression of pneumonia.[337] However, calves with moderate pneumonia (based on necropsy findings >10% lung consolidation) had significantly lower nasal planum temperatures compared to calves with mild pneumonia (<10% lung consolidation).[337]

Detection of lameness by use of IRT is of mutual interest in beef and dairy production. IRT is a unique diagnostic modality, as it can be rapid in localization of temperature differences in nonspecific lameness as well as performed from a distance in fractious animals, including bucking stock.[338,339] Expectedly, given weight distribution in cattle, evaluation of claw sole

temperatures demonstrates differences between front and hind limbs, as well as between medial and lateral claws - warmer temperatures in hind limbs compared to forelimbs, and in front medial and hind lateral claws compared to the contralateral claw.[340] Oikonomou et al. (2014) found sole temperature to increase as locomotion scores increased and digital cushion thickness decreased.[341] As reported by Wilheim et al. (2015), use of serial IRT assessment for the detection of sole hemorrhages associated with subclinical laminitis was not predictive, but rather temperature differences reflected only temperature differences associated with weight distribution.[340] In contrast, others have found IRT changes in skin and coronary band temperature capable of distinguishing healthy from abnormal claws.[342,343] Increased coronary band temperatures were associated with an increased incidence of sole hemorrhages.[343] Based on pre-trimming and post-trimming coronary band temperatures, lame claws had higher coronary band temperatures compared to normal claws ($P < 0.05$), whereas post-trimming claws demonstrated differences in skin, coronary band, and changes in temperature between lame and normal claws ($P < 0.01$).[342] Importantly, this may suggest that the cow can serve as its own control. With the aim of detecting foot lesions, coronary band temperature threshold values of 0.64ºC at 3 d pre-trimming (sensitivity 85.7% and specificity of 55.9%) and 1.09 ºC at 3 d post-trimming (sensitivity 80.8% and specificity of 82.9%) could be utilized.[342] Stokes et al. (2012) found a foot temperature of ≥27 ºC capable of detecting 80% of foot lesions correctly; however, differentiation of different foot pathologies based on temperature differences was not demonstrable.[344] Wood et al. (2014) were able to identify changes in foot temperature up to 6 weeks prior to the diagnosis of hoof lesions with an estimated prediction value of 0.623 ºC to identify affected feet.[345] Further, decrease in hoof temperature with resolution of lesions, 6 weeks post-treatment were observed.[345] Detection of infectious hoof diseases by IRT, such as digital

dermatitis is also possible. Alsaaod et al. (2014) found feet affected by digital dermatitis to have higher coronary and skin maximum temperatures compared to healthy feet ($P < 0.001$).[346] Skin temperatures increased with advancing stages of the disease.[346] Using a threshold temperature difference between rear and front feet of 0.99 ºC afforded a diagnostic sensitivity of 89.1% and a specificity of 66.6% for the detection of digital dermatitis.[346]

Temperature-based sensors are readily applied noninvasive tools for the objective quantification of temperature in cattle. Multiple modalities demonstrate effective measurement of surface, subcutaneous, and internal temperatures in cattle. In addition to use of temperature monitoring for the detection of estrus, use of these devices in combination may improve the detection of febrile responses during an APR and disease, under field conditions. However, several obstacles exist for implementation under commercial operations. Ease of application and retention need to be improved for thermometry sensors worn on the tail or placed intravaginal. Rumen boluses demonstrate excellent retention rates, but continuous temperature data relay is limited by the device's battery life and the need for proximity to a panel or antennae reader. Further research under controlled field settings to standardize data acquisition for hand-held and mounted IRT units is warranted, as variability in image acquisition lessens accuracy of skin temperatures obtained. To the author's knowledge, large scale commercialization of IRT cameras and software for use in commercial beef or dairy production has not been realized to date. However, knowledge of the modality in the industry is known, with its description found in lay publications.[347,348] Ideally, commercial automation of IRT units and use of computer software to analyze data will be carried out in the future.

Feeding and Drinking Behavior Monitoring

The sickness behaviors of anorexia and reduced thirst typically occur in conjunction with the febrile response. At first glance, this may seem paradoxical, as febrile animals need calories to fuel an increased body temperature, need to reduce the demand for muscle proteolysis, and to replace protein and other building blocks of tissues as a direct effect of disease. However, these sickness behaviors may serve a protective role, as discussed previously. From an objective, remote monitoring standpoint, a reduction in the motivation to seek food and water during sickness can be utilized by monitoring changes in eating and drinking behaviors. Several different modalities exist, including bunk attendance systems and rumination monitors.

Feed Bunk Attendance Systems

Early technologies capable of evaluating individual feed intake include Calan gates (American Calan, Northwood, NH)[f] and Pinpointer system (Universal Identification Systems Corp., Cookeville, TN). However, these systems require training of cattle to find and use feed stations, which can be time and labor intensive. Newer systems utilize electronic radio frequency identification (RFID), enabling the collection of behavioral data from large groups of animals with less labor compared to earlier methods and unencumbered feeding behavior in large groups of cattle. The GrowSafe System (Airdrie, AB, Canada)[g] is one such example which has been validated[349-351], with several other systems marketed for beef and dairy operation use (e.g. Lely™ Cosmix[h], FDS3 by S.A. Christensen & Co.[i], and Nedap electronic feeding stations[j]). Various RFID-based systems have been used to monitor feeding behavior including those that measure bunk attendance from feed alleys [352,353] and attendance from open[354] or gated[355] feed bunks. These systems monitor the frequency and duration of visits to the feed bunk (i.e. feeding time), with some systems capable of also quantifying the weight of feed consumed during each bunk visit when individual load cells are utilized (i.e. feed intake). Similar watering behavior

monitoring systems exist. Several research groups have validated these systems in both beef and dairy cattle production settings and have demonstrated a direct relationship of cattle feeding behavior with health and performance.[351,355,356] Given the importance of feed costs in production, use of bunk attendance systems in research and commercial settings have been utilized in the evaluation of feed efficiency and the related traits of average daily gain (ADG), dry matter intake (DMI), feed conversion ratio, and residual feed intake (RFI) in healthy cattle.[357-359] Investigation of reduced feeding and watering behaviors as indicators of illness in cattle is not new, as it is well known that fever associated with disease reduces feed intake.[17] However, use of remote technologies with the capabilities of individualized and real-time data acquisition are increasing in their application in research and commercial production settings.

Detection of sickness in feedlot cattle is important to limit the production and health impacts of bovine respiratory disease (BRD) and other morbidities in newly received animals. Recently shipped and received animals are subject to numerous stresses, including co-mingling, transportation, changes in feed, and exposure to various infectious agents. Use of continuous monitoring systems and the quantification of eating and drinking behaviors in newly received cattle provides sensitive and earlier detection methods, which are complementary to conventional methods of disease detection such as pen checking by experienced stockmen. Basarab et al. (1996) reported the use of the GrowSafe System to detect morbid feedlot animals 4 d earlier than conventional methods, with an accuracy of 81.5% for detecting BRD in recently placed steers.[360] These findings were based on changes in watering behavior under a commercial feedlot setting (Acme, AB, Canada), with morbid steers treated for BRD spending 23.7% less time at the watering station compared to healthy steers ($P < 0.001$).[360] Feed trials performed by Sowell et al. (1999) demonstrated greater number of feeding bouts ($P < 0.009$) and increased duration of time

spent feeding ($P < 0.001$) in healthy steers compared to moribund steers, with pronounced differences during the first 4 d on feed.[361] However, differences in drinking behavior were not found to be predictive of morbidity.[361] Differences in feeding and watering behavior in morbid versus healthy cattle are dynamic and can change throughout the feeding period, as demonstrated by Buhman et al. (2000). Frequency and duration of drinking were significantly greater 4 to 5 d after arrival in sick calves ($P < 0.05$), whereas the frequency and duration of eating and drinking were significantly lower 11 to 27 d after arrival in sick calves ($P < 0.05$), followed by a compensatory increase in the same behaviors between d 28 and 57 on feed.[70] Calves with minor or no pulmonary lesions at slaughter ate more often and for longer compared to cattle with severe pulmonary lesions, during d 11 to 27 after arrival.[70] This was in contrast to calves with severe pulmonary lesions, which ate significantly more often and for a longer duration later in the feeding period (d 28 to 57 days), likely reflecting differences in maintenance requirements or feed conversion, as well as rate of feed consumption in severely ill animals.[70] Quimby et al. (2001) were able to detect morbidity in 90% of sick feedlot cattle approximately 4.5 d earlier ($P < 0.001$) using cumulative presence and absence at the feedbunk compared to conventional methods typical of commercial feed yards.[352] An overall accuracy, positive predictive value, and sensitivity of 87%, 91%, and 90%, respectively, were reported for the identification of BRD using changes in feeding behavior compared to subjective assessment of calves by experienced feedlot personnel, based on simple linear statistics.[352] As reported by Wolfger et al. (2015), an increase in the mean intake per feeding, the mean time spent per feeding, the frequency of visits to the bunk, and interval between feedings was associated with a decreased hazard for developing BRD 7 d before clinical sign were observed ($P < 0.001$).[87] Use of nonlinear data mining analysis techniques, such as pattern recognition, clustering, and generation of algorithms,

in the evaluation of feeding behavior parameters captured by bunk attendance systems has been recently published.[362] With such techniques, the original variables obtained by automated feeding behavior monitoring system are combined into principal components (uncorrelated linear combinations of the original variables, also known as eigenvectors), and therefore differences in the original variables between healthy and morbid animals cannot be identified. Rather, based on the goal for disease identification, selection of the appropriate algorithm is carried out and applied to the data set.[362] Moya et al. (2015) developed several algorithms predictive of the health status of recently received heifers to a large commercial feedlot. Importantly, following the generation of such algorithms, these were subsequently tested on a naïve data set.[362] The BRD prevalence was 50% within this population of heifers, and 75% of the animals were identified as clinically ill within the first 14 d on feed.[362] Overall, correct prediction of health status was found in 4 out of 5 heifers, with a prediction accuracy of 79.2%, a precision of 100%, and a negative predictive value of 70.6%.[362] Most models demonstrated a high accuracy in predicting health status, and prediction of disease was typically at least 3 d prior to onset of clinically detectable signs.[362] In a group of 231 seed stock bulls, retrospective generation of models to identify changes in feeding behavior parameters predictive of BRD were carried out by Jackson et al. (2016).[363] Based on 30 of 231 bulls requiring treatment for respiratory disease, deflection in DMI was detectable 6.79 d before observation of clinical illness, with a reduction in DMI of 39.3% over the course of this period.[363] Change in feeding rate was detectable 1.32 d before clinical observation.[363] Other parameters included bunk visit duration and frequency of meals, which significantly changed 7.24 and 7.58 d prior to clinical detection, respectively, and preceded the reductions in DMI breakpoints by 0.45 and 0.79 d, respectively.[363] Accuracy of the non-feeding interval parameter was poorly predictive of disease onset.[363] In the remaining 201

bulls, which were metaphylactically treated with antibiotics at 38 DOF due to severe decrease in DMI in the group, parameters predictive of this change in feeding behavior included DMI, feeding rate, and bunk visit frequency.[363] In contrast to the ill cohort, feeding rate was one of the earliest traits detected, at 8.2 d before metaphylactic treatment.[363] Whereas change in bunk visit duration was not found prior to metaphylactic treatment, this parameter was identified in the ill cohort.[363]

Similar to feedlot cattle, utilization of feed behavior variables generated by bunk attendance systems allows for the identification of infectious and non-infectious diseases in dairy cattle and milk-fed calves. A negative impact on dry matter intake (DMI) has been demonstrated in association with reproductive issues (e.g., difficult calvings, retained placenta, and metritis), metabolic disorders (ketosis and milk fever), gastrointestinal disease (enteritis, displaced abomasum, and subacute ruminal acidosis), mastitis, and lameness. Furthermore, comparable to the detection of BRD in feedlot cattle, evaluation of feeding parameters in housed dairy cattle has been found to also be predictive of the onset of disease, leading to a more timely identification of cows at risk of post-partum disease, before observation of clinical signs.

Huzzey et al (2007) found prepartum feeding behaviors to be predictive of the onset of mild and severe metritis postpartum.[364] Cows that developed severe metritis spent less time feeding and consumed less feed compared to healthy cohorts, beginning 2 weeks before the observation of clinical signs of infection ($P < 0.05$).[364] For every 10-minute decrease in average daily feeding time during the week prior to calving, the odds of severe metritis increased by a factor of 1.72.[364] Furthermore, for every 1 kg decrease in DMI during the same period, cows were nearly three times more likely to be diagnosed with severe metritis.[364] Unlike the changes observed in feeding behavior, reduced water intake and time spent drinking were not as useful as

reduced DMI for prediction of metritis post-calving.[364] These findings are in agreement with the prior work of Urton et al. (2005), in which cows suffering from metritis spent, on average, 22 minutes less per day at the feed alley during the transition period compared to healthy cows.[353]

In a study by Gonzalez et al. (2008), cows diagnosed with clinical ketosis were found to have decreased DMI 3 d prior to the development of clinical signs, with a corresponding decrease in daily feeding time of 45 min per day.[365] Other disease states evaluated included mastitis, and acute and chronic lameness. In contrast to ketosis, acute locomotion disorders resulted in a small decrease in daily DMI before clinical diagnosis.[365] However, potential identification of acutely lame cows might include analysis of feeding rates, which were increased 2 to 3 fold from individual cow baseline.[365] Chronic locomotion disorders produced gradual changes in feeding behaviors, but cumulatively were similar to changes in feeding behaviors observed in acutely lame cows.[365] The use of feeding behavior alone may not be a suitable detection indicator for mastitis because not all types of mastitis identified in the Gonzalez et al. study lead to a significant reduction in feed intake. Feeding behavior should be used in conjunction with other detection modalities, such as milk yield and electrical conductivity.[365] Goldhawk et al. (2009) found DMI was reduced the week prior to calving in cows subsequently diagnosed with subclinical ketosis the first week post-partum.[366] Cows diagnosed as suffering from postpartum illness spent less time at the feed bunk, visited the feeder less often, and had decreased DMI in the week prior to calving[366], similar to Huzzey et al. (2007).[364] For every 10-minute decrease in average daily time spent at the feeder during the week prior to calving, the risk of subclinical ketosis increased by a factor of 1.9.[366] During the same week, a 1 kg decrease in average daily DMI increased the risk of subclinical ketosis by 2.2.[366] Although not significant

($P = 0.07$) cows which developed subclinical ketosis also had reduced water intake during the 2 weeks prepartum.[366]

There is growing interest in the use of group housing in preweaned dairy calves.[367] Group housing of preweaned calves poses special problems because it may be difficult to ensure appropriate milk intake by all individual calves. The need for monitoring individual milk intake has led to an increase in the use of automatic milk feeders. Automatic milk feeders typically offer calves a daily-allotted ration in 0.5 to 2 L portions over several meals in a 24h period. Important factors with the use of automatic feeders including number of calves per feeder, milk allowance, and milk flow rate; these factors will affect competition amongst calves, the number of unrewarded visits to the feeder, and cross suckling behaviors.[368-370] As with bunk attendance systems in older cattle, use of data generated from automatic milk feeders has demonstrated the ability to identify illness in preweaned calves based on milk feeding behavior.[371-373] Commercially available automatic milk feeders offer alarm lists based on milk consumption, and some offer alarm functions based on drinking rate. However, the parameters provided by automated milk feeders best able to detect illness in calves varies between studies. When milk-feeding behaviors are used for the detection of disease, the amount of daily milk allowance is especially important. Maatje et al. (1993) evaluated milk intake, growth, and detection of illness in veal calves raised in group housing with automated milk feeding, compared to bucket fed, individual housing conditions. Pertinent to the detection of morbidity in veal calves fed large amounts of milk, drinking rate followed by total milk consumption were found to be the most sensitive indicators of disease status.[371] Frequency of unrewarded visits was not associated with disease status in veal calves fed high milk levels.[371] The majority of calves eventually treated for illness demonstrated reduced feeding behaviors within 7 d before clinical signs were detected,

with only 17.5% of treated morbid calves showing no changes in feeding behaviors.[371] Addressing the effect of milk allowance on disease identification, Borderas et al. (2009) found that sick calves fed high allowances of milk (8 L per day) decreased their milk intake and frequency of visits to the milk feeder, and increased the duration of each visit to the feeder compared to healthy calves at the same milk allowance ($P < 0.05$).[373] Sick calves fed a low milk allowance (4 L per day) had no difference in milk intake or frequency of visits to the feeder ($P = 0.6$ and 0.2, respectively) compared to healthy calves at the same milk allowance.[373] But, a reduced duration of visits to the milk feeder on the day of illness was detected and on the following 3 days ($P < 0.01$), compared to healthy calves at the same milk allowance.[373] In contrast, Svensson et al. (2007) found only the frequency of unrewarded visits to the milk feeder useful for the identification of morbid calves, whereas none of the other parameters were significantly associated with disease.[372] In that particular study, calves were on a restrictive milk allowance (6 L per day), and a lower level of unrewarded visits were observed in sick calves.[372] The ability to predict the occurrence of disease prior to its onset in preweaned, housed dairy calves based on milk feeding behaviors is varied in the literature. Changes in milk feeding behavior which preceded the detection of clinical signs has been demonstrated in some studies[371,372], but not all.[373] Feed intake parameters in group-housed dairy calves are subject to multiple variables, as well as differences between disease severity among studies, which likely accounts for some of the disparity.

From a health and welfare standpoint, the use of bunk attendance systems and automatic milk feeders in weaned cattle and calves, respectively, have demonstrated effectiveness in the detection of morbidity associated with several diseases of cattle, under commercial conditions in the dairy and beef sectors. Further research is needed to refine feeding parameters measured and

analyzed, which may improve the efficiency and accuracy of disease detection, earlier in its onset, as well as improving the ability of eating and drinking behaviors to predict cattle at risk for becoming ill. Future research will also have to demonstrate strong economic justification for the costly implementation of such bunk and water attendance systems on commercial operations.

Rumination Activity Monitoring

Pressure-based Rumination Sensors

Non-invasive, wearable sensors for the quantification of mastication and deglutition behaviors are described in humans and in veterinary species, including rumination patterns in livestock. Monitoring of rumination activity serves as a measure of rumen health and well-being, with demonstrable relationship between rumination and feeding and lying behaviors.[374] Both strain and pressure-based gauges have been utilized to capture jaw movements of chewing and motion of the larynx with swallowing. Acoustic methods can detect sounds characteristic of chewing, swallowing, and regurgitation. In livestock, the early work of Law and Sudweeks (1975) describes monitoring of rumination in cattle by a simple, halter-mounted system for the capture of jaw movements (pneumatic, pressure-based data), transformed into an electronic signal and converted to chewing rate.[375] Beauchemin et al. (1989), describe the use of halter-imbedded transducers (two strain gauges bonded to a strip of spring steel, formed into a concave shape), which relay signals corresponding to jaw movements.[376] An algorithm was developed to process the digital signal into discrete chewing periods, including differentiation between eating and rumination.[377] Complex spectral analysis, as introduced by Brillinger (1973), was used to decompose the complex patterns into their component cycles; these cyclical, complex patterns overlay a circadian rhythm in the rumination patterns of four tie-stall housed Holstein cows.[377] Early use of these indirect methods of monitoring rumination, based on jaw-motion detection are

effective in quantification of rumination patterns, however, the animal is required to wear a halter, and the devices were relatively cumbersome in their application and use (e.g. wires and tubing associated with signal transduction, limited to tie-stalled animals), and were limited in their storage capacity.

More recently, evaluation of a pressure-sensitive sensor mounted on the noseband of a halter (MSR-ART; Agroscope Reckenholz-Tanikon ART, Ettenhausen, Switzerland)[k], has been evaluated. In Braun et al. (2013), 10 tie-stalled Brown-Swiss cows were evaluated for rumination activity using the noseband sensor compared to visual observation, over a 24 hour period.[378] Eating and rumination were easily distinguishable based on pressure profiles generated.[378] Strong correlations between the two recording methods were found for eating, ruminating, and resting times, as well as the number and duration of eating, ruminating, and resting phases.[378] Agreement between the pressure sensor data and direct observation was approximately 98.8%.[378] In continuance, Braun et al. (2014) evaluated the noseband pressure sensor over 10 d in the group of animals. Calculation of the coefficients of variation (CV) ranged from 5.9 to 12.7%, with rumination CV (chewing cycles per bolus; daily number of cuds; rumination time) being smaller compared to eating (eating time; chewing cycles related to eating).[379] On a larger scale, the same group established reference values for the rumination sensor for three dairy breeds (i.e. Brown Swiss, Holstein-Friesian, and Swiss Fleckvieh), typical of Swiss dairy production.[380] Pahl et al. (2016) report the suitability of the noseband sensors as an estimated measure of feed intake.[381] Using a bunk attendance system to quantify feeding time and consumption, the level of accordance between the two systems ranged from 89.6% to 94.1%.[381] Feeding time (bunk-based data) was more accurate, with a correlation of 0.891 between feeding time and DMI, compared

to chewing time, with a correlation of 0.780; however chewing time per bout was still found to be a suitable measure of feed intake.[381]

Use of optic fiber Bragg grating (FBG) sensors for evaluation of mastication and swallowing disorders has been described in human medicine. To date, this is very limited in its application to cattle. Pegorini et al. (2015) report the use of FBG sensors for the classification and quantification of eating patterns in cattle.[382] Present application of these sensors requires surgical implantation, which will limit the device in large field trials.

Acoustic-based Rumination Sensors

Whereas the devices described above utilized pressure-based signals associated with jaw muscle movements, another type of quantifiable signal is sounds associated with regurgitation, mastication, and swallowing for measurement of rumination activity. For example, the Hi-Tag rumination monitoring system (SCR Engineers Ltd., Netanya, Israel)[l] consists of a rumination logger (microphone) worn as a collar on the left side of the neck, a stationary or mobile reader, and software for processing the electronic data. Sounds unique to regurgitation and rumination, respectively, are recorded, processed, and digitally stored. Two-hour blocks of data are summarized, with a storage capacity of the device limited to 22 h. Rumination time is presented as either minutes per 2 h block or minutes per day. Data are downloaded via readers, which can be hand-held or strategically positioned within the barn (e.g. water trough) or milking parlor. This system is integrated in commercial activity tags (e.g. Hi-Tag which are called HR-Tag, SCR Engineering Ltd.[l] or the Qwes-HR Tag as part of Lely's Astronaut A4 milking robot system[m]).

Based on concurrent visual observation as the reference standard, acoustic rumination activity monitors have been validated under various production settings and in different classes

of cattle. As reported by Schirmann et al. (2009) in individually penned and small group-housed dairy cattle, strong correlations (r = 0.93, $P < 0.05$) were found between the HR-Tag sensor data and human observation.[383] Ambriz-Vilchis et al. (2015) also report good agreement under housed conditions; however, rumination collars performed poorly in grazing dairy cattle.[384] Burfeind et al. (2011) report evaluation of the Hi-Tag system in young calves (46 ± 14 days) and heifers (208 ± 78 d).[385] A moderate correlation between sensor and human observation of rumination activity was found for animals greater than 9 months of age (r = 0.88); but, a great variation in the agreement was found for calves less than 9 months of age (r = 0.47 to 0.89).[385] Evaluation in beef cattle, by Goldhawk et al. (2013), found rumination activity only moderately correlated with visual observation (r = 0.41, $P < 0.001$), with the Hi-Tag system[l] generally underestimating rumination and suggesting the system to be relatively inaccurate for beef cattle on finishing diets.[386] Elischer et al. (2013) evaluated activity monitors incorporated into automatic milking systems (AMS) under pasture-based conditions.[387] Cow behavioral activities were quantified using the Lely transponder collar, which houses both an activity monitor (i.e. accelerometer) as well as a rumination monitor (Qwes-HR Tag, Lely)[m]. Additionally, cows wore a pedometer (IceQube, IceRobotics, Edinburgh, UK)[n]. The activity monitor worn about the neck was inferior in accuracy to the behavioral descriptions produced by the pedometer, especially in capturing postural behaviors such as lying.[387] The correlation between rumination sensor activity and human observation was significant (r = 0.65, $P < 0.05$), but the authors felt it to be unacceptable and likely due to malfunction or misplacement of the collar-worn sensor.[387]

In free-stall housed dairy cattle, Borchers et al. (2016) evaluated several commercially available activity monitors, including the rumination monitors of Smartbow (Smartbow GmbH, Jutogasse, Austria)[o] and CowManager SensOor (Agis, Harmelen, Netherlands)[p], compared to

human observation.[388] Visually recorded rumination behaviors were strongly correlated with the Smartbow ($r = 0.97$, $P < 0.01$), but weakly correlated with the CowManager SensOor ($r = 0.69$, $P < 0.01$).[388] Importantly, the two ear-tag sensors evaluated use accelerometer-based data for the quantification of rumination activity, as opposed to jaw movements or eructation sounds. Results of Borchers et al. (2016) demonstrate that some accelerometer-based sensors for the quantification of rumination activity may underperform compared to other modalities.[388] This is in contrast to the pilot study by Bikker et al. (2014), which also evaluated the CowManager SensOor in dairy cattle, housed in free-stalls.[389] Correlation between human observation and sensor data for rumination activity was strong ($r = 0.93$, $P < 0.01$).[389]

Factors that affect rumination time include cow-specific, dietary, husbandry, and environmental conditions. Primiparous cows demonstrate shorter daily rumination times compared to multiparous cows, likely due to reduced total daily feed intake in younger cows, although frequency of meals and duration of feeding per day tend to be greater.[390] Changes in environment and social hierarchy, such as the introduction of newly freshened primiparous cow to the lactating string, have an impact on rumination time. Schirmann et al. (2011) observed a reduction in rumination time after regrouping, especially pronounced in heifers.[391] A number of nutrition-based studies evaluating the impact of NDF, fiber length, DMI, and other measures on rumen health are reported, with several specifically evaluating rumination activity.[392] Byskov et al. (2015) assessed the impact of large variations in dietary contents on rumination time in free-stall housed dairy cows (acoustic neck collar; RMS RuminAct-Milkline, Gariga di Podenzano, Italy)[q], taking into account parity, stage of lactation, and level of milk production.[393] Rumination time was positively related to intake of forage NDF and starch, whereas intake of sugar and remaining fraction were negatively related to daily rumination time.[393] Rumination time, in

minutes per kilogram of DMI, was negatively related to milk yield and protein percentage, and positively related to milk fat percentage.[393] Approximately 32% of the variation in daily rumination time was explained by variations in intakes of the dietary fractions, whereas the remaining 48% was due to individual variations between cows.[393] Although rumination time was indicative of forage to concentrate ratio at the group level, caution is warranted for the use of rumination time in predicting individual cow feed intake. However, rumination times collected serially over extended duration may be useful for monitoring the relative feed intake in individual cows.[393] In contrast, work by Stone et al. (2017), found positive correlations between rumination time and milk yield, purportedly due to greater-yielding cows needing to eat more and subsequently ruminate more often.[394]

Use of rumination monitors for the detection of estrus behavior in cattle is commonplace on dairy operations utilizing precision farming activity monitors. A study by Pahl et al. (2015) describes feeding characteristics and rumination time changes useful for the early detection of estrus in free-stalled dairy cattle.[390] Significant reduction in rumination time was detected on d -1 ($P = 0.037$) and d 0 ($P = 0.044$), with day of insemination assigned d 0.[390] Reductions in rumination time were approximately 75 min compared to baseline, in both primiparous and multiparous cattle.[390] Rumination time nadirs were on d -1 and d 0 for multiparous and primiparous cows, respectively, and returned to baseline by d 1.[390] These findings are similar to those of Reith and Hoy (2012), wherein rumination time was significantly reduced in cows during estrus (classification based on visual observation of estrus behaviors).[395] Minimum rumination times were observed on the day of estrus in both primiparous and multiparous cows.[395] The average decrease in rumination time was 17%, with greater percentage decreases observed in primiparous cows compared to multiparous cows.[395]

Like changes in feeding time and locomotion activity, changes in rumination activity may also be predictive of calving onset. A study by Soriani et al. (2012) reports changes in rumination time on the day of calving, but did not report changes relative to the time of calving.[396] More recently, others have evaluated changes in rumination activity with reference to an established calving time. Schirmann et al. (2013) found cows spent, on average, 63 ± 30 min/24 h less time ruminating during the 24-hour period before calving compared to baseline.[397] Using 2-h blocks of data collection, changes in rumination activity started to decline approximately 4 h before calving and started to increase towards baseline by 6 h post-partum.[397] In Clark et al. (2015), decreases in rumination time averaged 15% from d -2 to -1, with a further decrease of 18% from d -1 to the day of calving; thus, representing a reduction of 33% in rumination time within 2 d of parturition.[398] Using a threshold value of 0.9 (a decline in rumination by 10%), 70% sensitivity and specificity were observed for prediction of calving within 24 h.[398] Buchel et al. (2014) report significant reductions in rumination time 6 h prior to onset of parturition, with an average decrease in rumination time of 27%.[399] Similarly, Pahl et al. (2014) observed several decreases in rumination activity within 24 h of calving.[400] Reduction in the number of jaw movements during rumination bouts and the number of cuds masticated per day were decreased in the 24 h prior to parturition compared to baseline.[400] However, significant reductions in rumination time were observed only in the last 4 h prepartum and in the first 8 h postpartum.[400] Although a trend for a longer hiatus between the final rumination bout and calving compared to preceding rumination bouts was found ($P = 0.059$), the duration of time between cessation of rumination and calving was highly variable between cows, ranging from 17 to 220 minutes.[400]

The literature supports the use of rumination time for the early detection of cows at risk or currently experiencing illness during the puerperium. In Soriani et al. (2012), changes in

rumination time were both predictive of calving as well as the presence of subclinical disease or health disorders post-partum.[396] Cows demonstrating decreased rumination time during the first 10 d post-calving had a greater incidence of mastitis, lameness, ketosis, and displaced abomasum during the first 30 d of lactation.[396] In addition, decreases in rumination time in moribund cattle corresponded to a marked acute phase response compared to mild inflammatory changes associated with the puerperium in healthy cows.[396] As a marker of animal welfare, Soriani et al. suggest post-partum cows within the first 10 d of lactation should demonstrate rumination times of >550 min/d.[396] Likewise, Calamari et al. (2014) describe the associations between rumination time, severity of inflammation and the presence of post-partum disease.[401] Cows showing high rumination times pre-calving rapidly returned to high rumination times between 3 and 6 d after calving, whereas cows with low rumination times pre-calving were slower to return by 15 d in milk.[401] Interestingly, > 90% of the cows in the low rumination time group were diagnosed with a post-partum disease, with only 42% of high rumination time cows experienced post-partum disorders.[401] Greater increases in APP and other markers of metabolic disease (e.g. NEFA) were observed in the low rumination time cows, reaching significance between low and high groups by d 10 of lactation.[401] However, the relationship between markers of the APR and rumination time was poor beyond 10 d post-calving.[401] Additionally, inclusion of an absolute rumination time threshold (>550 min/day) to indicate health status was not valid in this study.[401] As expected, absolute rumination time would be a poor predictor, rather than trends and percentage changes from baseline, given different seasonal, environment, and dietary conditions are found between dairy operations and across studies. In Kaufman et al. (2016), associations between rumination time and subclinical ketosis (and other disorders) were demonstrable in multiparous dairy cows.[402] Cows with hyperketonemia (BHB > 1.2 mmol/L) and cows with hyperketonemia

and concurrent diseases (e.g. retained placenta) had decreased rumination by 25 ± 12.8 min/day and 44 ± 15.6 min/day, respectively, compared to healthy cows.[402] Differences in rumination time were appreciable between health groups during the 7 d preceding and 14 d following calving.[402] A large field trial by Stangaferro et al. (2016), reports the use of a composite health index score (HIS), which combines rumination time and activity indices, for the detection of cows with metabolic and digestive disorders, as well as mastitis and metritis.[403-405] Proprietary algorithms (SCR Engineers Ltd., Netanya, Israel)[l] generated a HIS of 0 to 100 with arbitrary units, with a score of less than 86 indicative of a health disorder. Farm personnel, blinded to HIS results, performed daily clinical assessments of cows from 1 to 10 DIM, which included physical examination, rectal temperature, and urine ketone assessment. Additionally, pre- and post-calving serum biochemical analyses were performed. Except for indigestion, the sensitivity of HIS to detect cows with ketosis, metabolic disorders, and displaced abomasum was greater than 90%, and cows were identified on average 3 d earlier based on HIS compared to clinical detection by farm personnel ($P < 0.01$).[403] The sensitivity of HIS was 58% for all cases of clinical mastitis, and 55% and 89% for cases of mastitis alone or concurrent with other health disorders, respectively.[404] Of clinical mastitis cases caused by *E. coli*, the HIS had a sensitivity of 80.7%.[404] Similarly, the HIS sensitivity for the detection of metritis was 55%, 53% for metritis alone, and 78% metritis concurrent with other disorders.[405] Overall, the HIS had a very high negative predictive value of 98%, indicating it was effective in identifying cows with severe cases of metritis, but was less effective for the identification of mild cases.[405]

Similarly, Steensels et al. (2016) report modeling capabilities of data captured by the HR-Tag® monitoring system (SCR Engineers Ltd.[l]) for the detection of post-partum diseases in commercial dairy cattle.[406] Three-hundred healthy and 403 sick cows were included, with the

classification of four disease categories: healthy, ketosis, metritis, and lameness. Rumination time in cows with ketosis and/or metritis was lower during the period from 5 d before until 2 d after diagnosis and treatment compared to rumination activity of healthy cows, with the greatest difference in rumination time observed 3 d prior to clinical detection (7.5 ± 0.5min/2h, $P <$ 0.001).[406] Furthermore, differences in accelerometer-based activity and milk yield were observed from 5 d prior to disease detection, with the greatest differences in these modalities observed 1 d prior to clinical detection.[406] Steensels et al. (2017) also evaluated the HR-Tag® monitoring system (SCR Engineers Ltd.[l]) for the detection of ketosis using logistic (binary) regression modeling.[407] Performance of the described model detected ketosis 1 d before detection on routine health examination, with an average accuracy of 76%.[407] When the model was calibrated based on data from the same farm, sensitivity could reach 90%; however, dependent on the data set, the model's sensitivity and specificity ranged from 0.78 to 0.90 and 0.71 to 0.74, respectively.[407]

Utilizing rumen bolus technology, Nogami et al. (2017) have recently described the development of a rumen bolus accelerometer and thermistor prototype, the former attribute designed to detect changes in rumen motility and alert to the presence of rumen atony.[408] The prototype demonstrated promise in distinguishing between normal rumen motility and atony (pharmacologically induced). However, the pilot study was performed using one animal, and activity was limited by tie-stall housing.[408] Further evaluation in a larger group of cattle, under loose housing, is required to determine the ability to detect and distinguish based on algorithm generation, rumen acceleration data from locomotor activity data.

Acoustic-based rumination monitors offer several strengths, including the ease of application and retention of devices worn on a neck collar when compared to necessity of wearing a pressure-based sensor on a halter. The later would be severely limited in its application

on most large dairies, and undoubtedly on most commercial beef operations. Furthermore, inclusion of other activity monitors, most often an accelerometer-based sensor, affords the collection of several types of data concurrently, typically implemented on farm as a tool in reproductive management and estrus detection. Both pressure and acoustic based sensors provide complementary data to bunk attendance systems. The latter are more suitable for generating parameters of DMI and feeding period, with rumination activity providing different parameters equally reflective of rumen activity and overall health. Similar to the bunk attendance systems, use of rumination activity monitors has proven useful for the detection and prediction of disease in cattle. Areas of further research include the refinement of these technologies for use in younger classes of cattle. Solutions for the feasible implementation of these devices under extensive pasture conditions as well as in the feedlot are also needed.

Monitoring Posture and Locomotion Behavior

Accelerometers

Characterization of locomotor and postural activities, such as walking, standing, and lying, are described for several domestic species, including beef and dairy cattle. A large body of research has been performed in dairy cows evaluating pedometers and accelerometers primarily for the purpose of estrus detection. More recently, algorithms for activity-monitor data analyses for the detection of other physiological and pathological states, including sickness behavior in cattle, have been developed. Application of accelerometers was the remote monitoring technology used herein, in both experimental subclinical disease models as well as in healthy animals during the peripartum and neonatal periods. In brief, accelerometers are small, non-invasive devices used to for objective monitoring of static postural and dynamic locomotion behaviors.[409] Accelerometers capture triaxial measures of acceleration of either the distal limb or

head and neck, dependent on location of device and algorithm used for data analysis. This allows quantification of time spent standing, lying, and walking, as well as frequency of lying bouts and steps. The device does not measure acceleration continuously but rather takes point measures of acceleration at a predetermined rate (i.e. sampling rate or epoch).[409] Typically, devices are worn on a neck collar or as a leg-band on the distal metatarsus.[l,m,n] More recently developed devices include ear-tag based accelerometers (e.g. SensOor, CowManager®, Utrecht, Netherlands)[r].[410] Commercially available activity monitors often contained multiple sensors for capture of different data modalities. Other methods of determining activity include the use of a triangulation positioning system, whereby changes in the sensor's coordinates, within a defined area, are used to measure distance traveled, as well as apparent proximity to locations (e.g. feed bunk, water trough, or shelter).[411,412] Devices used for tracking wildlife or domestic livestock include devices incorporating global positioning systems, accelerometers, and magnetometer capabilities.[413] Validation of accelerometer-based data for characterization of behavioral activity indices in dairy and beef cattle, under various management conditions, has demonstrated a high level of agreement and accuracy to human observation and video surveillance data.[388,409,414-416] However, differences in sensitivity and specificity for the behavior indices exist between different brands of activity monitors. [387,388,417] In general, accelerometers tend to be more accurate for static behaviors compared to dynamic behaviors, although this is highly dependent on sampling frequency.[418] Depending on the specific behavior evaluated, unacceptably high error rates can occur. This is not necessarily due to the device or data, but rather the inherent complexity of classifying composite behaviors. Ungar et al. (2017) report a 22% classification error between grazing and non-grazing, as differentiation between upright resting and grazing was difficult.[419] Use of ear-tag activity sensors shows acceptable accuracy for resting behaviors and rumination

monitoring, whereas agreement for activity was only moderate.[389] An important aspect to consider is the use of activity monitors in classes of livestock different from those validated, such as young calves compared to adult dairy cows. As reported by Trenel et al. (2009), accelerometers were accurate for lying and standing behavior in calves, whereas walking behavior was inaccurately overestimated based on a sampling rate of 8 readings per second.[420] In contrast, de Passille et al. (2010) used a high sampling frequency (33 readings / second) for accurate step count and differentiation of gait pattern in young calves.[421] Bonk et al. (2013) found strong correlation for lying time and bout frequency with observation in calves if a sampling time of ≤ 60-sec was used.[422] Similarly, strong correlations (r = 0.99) between accelerometer-based data and video recordings for step activity, lying bouts, and lying time are reported by Swartz et al. (2016) in young calves.[423]

Given the importance of reproductive management, initial implementation of activity monitoring principally focused on the use of behavioral data for the detection of estrus on commercial cattle operations. Redden et al. (1992) report pedometer-based activity monitoring for the detection of estrus in 25 tie-stalled dairy cows.[284] Step counts were retrieved twice daily at milking. Data underwent a two-step process for determination of a positive threshold, whereby an increase in activity >50%, based on a minimum of 5 d baseline and activity increase not occurring within 15 days of last spike.[284] Mean total daily activity was increased 2.3-fold at estrus ($P < 0.0001$), with the majority of increased activity observed during daytime turnout from tie-stall confinement.[284] Activity monitoring resulted in an 80% estrus detection rate, with four false positives (16%). This was similar to estrus detection rate based on change in vaginal temperature in the same study, and both remote monitoring methods were superior to casual observation (54% detection rate).[284]

Commercial activity monitoring systems require dedicated software as part of farm-management systems, with algorithms for detection of estrus (or other health events) generally proprietary property of the manufacturer. Lovendahl and Chagunda (2010) developed an algorithm for the detection of estrus using the neckband activity tags from DeLaval (Alpro, version 6.60, Kansas City, MO)[s].[424] Therein, hourly-obtained activity data established an individualized hour-by-hour reference value; a relative increase from baseline, defined as three consecutive readings exceeding baseline, defined the initiation of a high-activity episode; and, episode strength, duration, and regularity were determined.[424] The optimization of thresholds provided an estrus detection rate of 74.6% and 1.3% daily error rate.[424] Cow-factors significantly affecting activity measures during estrus included breed, age, and parity.[424] During estrus, strength of activity was higher and more regular compared to the activity before and after the episode ($P < 0.001$).[424]

Similar, Aungier et al. (2012) evaluated activity monitor based estrus detection with concurrent sampling of systemic hormones.[425] SCR Heat-Tag (Heatime; SCR Engineers Ltd.)[l] is an activity monitor used in pastured dairy cattle. 2-h blocks of activity indices were used, with average of an 8 h data set (as downloaded at each milking) compared to a 7-d baseline. The Heatime system identifies an animal in a preovulatory follicular phase if the current average activity level was equal to or greater than 5 standard deviations above baseline. Based on manufacturer settings for cluster thresholds, Heatime successfully detected 72% of preovulatory follicular phases, missed 28% of preovulatory follicular phases, and incorrectly identified 32% of activity clusters that occurred during high-progesterone phases.[425] Preovulatory follicular phase activity clusters associated with subsequent postpartum ovulations had higher mean peak activity levels and mean durations compared to those occurring with the first post-partum ovulation.[425]

Clusters associated with high progesterone levels, thus representing false positives, had lower mean peak activity levels and durations than those activity clusters detected in periods of low progesterone and associated with the preovulatory follicular phases (true positives).[425] Furthermore, differences in peak activity and duration patterns were significantly different for all 4 endocrine states evaluated.[425] Activity clusters of borderline intensity and short duration, representing luteal states, were similar to those described by Lovendahl and Chagunda. Peak activity level was strongly correlated with duration ($r = 0.86$, $P < 0.0001$).[425] Based on ROC analysis, peak activity intensity of 13 standard deviations demonstrated a 79% sensitivity and 93.3% specificity for the detection of a preovulatory follicular phase (with only 6.7% of luteal phase falsely identified as preovulatory follicular phases).[425] The authors suggest that the Heatime system should use a 6 to 8 h duration threshold and maintain the borderline peak activity threshold to retain the largest number of preovulatory follicular phases possible.[425]

Based on a convenience sample of three free-stall commercial dairy herds in Ontario, Canada, Neves et al. (2012) evaluated the herd management systems based on remote modalities of activity monitors and mounting activity devices compared to timed artificial insemination (TAI) programs, using reproductive performance parameters.[426] Overall, herd pregnancy rate and cow-level time to pregnancy did not differ between TAI and activity monitoring management programs. However, an interaction between herd and treatment was observed, with 2 of the 3 herds demonstrating improved reproductive indices in that activity-monitored cows became pregnant sooner compared to TAI and the median time to first service shorter in activity cows compared to those enrolled in TAI programs.[426] The effect of treatment group on conception risk did not depend on herd.[426] Importantly, an increase in insemination rates based on false activity

data nor a reduction in conception rate with improper insemination timing were not observed in the herds using remote technologies.[426]

There are few reports evaluating the head-to-head comparison of multiple activity monitors concurrently on the same animal. Chanvallon et al. (2014) evaluated three automated devices: the AfiTag PM (leg pedometer; Afimilk®, Kibbutz Afikim, Israel)[t], Heatime-RuminAct (SCR Engineers Ltd.)[l] and HeatPhone (AM2; Medria, France) accelerometers worn on same neck collar. Classification (correct / incorrect) identification of estrus was based on milk progesterone concentrations. Pedometer monitoring had a sensitivity and positive predictive value of 71%, whereas lower sensitivity of approximately 60% were observed for both accelerometers, with positive predictive values ranging between 84 – 87%.[427] Also using multiple automated devices, Dolecheck et al. (2015) described the changes exhibited by cows during estrus. Secondly, machine-learning techniques using the automatically collected data were evaluated. Machine learning accuracy for all technologies ranged from 91 to 100% (compared to 66% for visual observation), and thus machine learning techniques could hold potential for estrus detection if applied to automatically collected technology data.[428] Although most remote monitors perform better than visual appraisal, this is not always the case. For example, Talukder et al. (2015) evaluated several remote technologies (IRT, rumination and activity data) for estrus detection in pastured dairy cows.[326] Based on the default thresholds set by the manufacturer, SCR HR LD tags (SCR Engineers Ltd.)[l] demonstrated a 78% Se, 57% Sp, and 70% PPV for the detection of estrus.[326] ROC analysis of combination of rumination and activity measures improved values to 82, 54, and 75%, respectively. However, these systems did not outperform visual assessment for estrus behaviors (75, 100, and 100%, respectively).[326]

Given the importance of sound animal husbandry and welfare, recent research has focused on the use of activity monitor data in housed dairy cattle to define the impact of cow- and herd-level factors, including routine husbandry practices and environmental conditions. Evaluation of time budgets for lactating dairy cows is important, as prolonged time spent away from the pen for milking and transit can have a potentially negative impact on lying time, feeding time, and disruption of normal social interactions. Use of various activity monitors allows objective quantification of these behaviors, as well as those associated with specific tasks, such as feed delivery, milking, and cow-flow through automatic milking systems. Simple, yet important questions known to be of importance can be validated with these remote technologies. For example, whether alterations in feed delivery timing and frequency relative to milkings affects activity indices and feed intake in intensively housed dairy cattle, with detailed descriptions of cow activity, using activity monitors and bunk attendance systems and the impact of frequency of feed delivery described in free-stalled dairy cattle.[429] Group-housed cattle activity in automatic milking systems (AMS, i.e. robotic milker), reported by Deming et al. (2013), demonstrated the importance of number of AMS units, bunk space allowance, and an increased frequency of feed push-up in order to maximize milking frequency and daily lying duration in this system.[430] Use of activity monitoring and the impact of feed delivery frequency has been quantified in cows milked thrice daily.[431] As reported by Devries et al. (2005), cows increased feeding time by 26% when fresh feed was made available upon return from milking compared to when it was not, whereas delivery of feed 6 hours after milking further increased feed intake by 12.5%.[432] This suggests that the delivery of fresh feed is a stronger stimulus for feed intake than the return from milking in tie-stalled dairy cows.[433] However, a recent study by the same group found opposing results.[434] Timing of feed delivery and its influence on post-

milking standing time is important from the perspective of new intramammary infections. Generally, it is held that promoting longer standing times post-milking reduces the risk of intramammary infection, by allowing sufficient time for teat canal closure. Feed delivery timing alters the time to first lying post-milking in tie-stalled dairy cows, as demonstrated by DeVries et al. (2010), therein a 1.4 risk reduction of intramammary infection was observed if lying time occurred between 40 and 60 minutes post-milking compared to those lying down within 40 minutes of milking.[435] However, as post-milking standing time increased past 60 minutes, the odds of acquiring a new infection increased.[435] Similar findings have been demonstrated in free-stalled cows using automatic milking systems.[436] Although not a direct comparison of parameters, in contrast Watters et al. (2013) demonstrated that first lying times at greater than 90 minutes post-milking decreased the risk of acquiring elevated somatic cell counts compared to less than 90 minutes, using activity monitors to quantify behaviors on five commercial free-stall dairy farms.[437] Novel algorithms for evaluation of activity monitoring data alone or in combination with other modalities have allowed refinement of time-budgets, such as quantifying time spent in transit to and from milking parlors at the pen and individual cow level.[438] Further use of accelerometer-based data to critically appraise management practices, improve cow-comfort and time budgets are areas of on-going research.

As discussed, the peripartum period represents a critical period during the cows reproductive life, in which remote, objective quantification of physiological and behavioral changes through the application of remote technologies could provide valuable information of impending parturition and potentially alerting personnel of the need for intervention during dystocic calvings. Quantification of behaviors in peripartum cows using video surveillance describes the characteristic changes associated with stage two labor, with increasing restlessness,

tail raising, and an increase in frequency of transitioning from standing to lying.[439-441] Use of remote technologies, including accelerometers, minimizes human observation required to review videotape footage. Use of activity data and the development of algorithms predictive of impending parturition is a recent application of these activity-monitoring technologies. Huzzey et al. (2005) demonstrated significant changes in mean daily standing time and an eighty-percent increase in the number of transitions from lying to standing during 24 h prior to and 24 h post-calving period in free-stall housed transition cows.[442] Recently, Titler et al. (2015) have developed an algorithm for the prediction of calving, based on observed calvings and activity data in dairy cattle.[443] Distinct behavioral patterns were demonstrated by both primiparous and multiparous cows, which included an increase in step count, increased standing time, decreased lying time, and increased frequency of lying bouts.[443] Using the activity index, which placed a greater significance on lying bout frequency compared to other behavior data, a significant change in activity was observed on average 6 h before calving (with a range of 2 to 14 h); and, in almost 76% of animals within 4 h of calving.[443] However, variability in the sensitivity and specificity for accelerometer-based activity data (and respective algorithms applied in analyses) for the prediction of calving is present in the literature. In part results are influenced by sample size and whether performed under controlled experimental conditions or field settings. For example, Rutten et al. (2015) and Borchers et al. (2017) report sensitivities ranging from 90 to 100% and specificities approximately 90% in a limited number of cattle.[444,445] In contrast, Santegoeds et al. (2016) report a sensitivity and specificity <30% with poor performance (0.82, based on AUC on ROC) not suitable for commercial use.[446] Although changes in activity, rumination, and ear temperature were demonstrated using multiple sensors, Rutten et al. (2017) similarly report poor performance of sensor data to predict calving onset, with an unacceptably

high false positive rate.[447] Depending on the window of prediction (e.g. exact hour, 6 h, and 12 h) assigned to the model, sensitivity ranged from 21.2 to 51.5%, with a fixed false positive rate of 1% (specificity of 99%).[448] The studies by Santegoeds et al. and Rutten et al. were based on 3,000 and 417 calvings, respectively, on commercial Dutch dairies.[446,448] Although significant changes in behavioral indices were consistent with previous studies, these studies illustrate the need for further research in the validation and refinement of activity-based data algorithms under field commercial settings for the prediction and accurate detection of calving.

Continued refinement of algorithms applied to behavioral activity data will promote further implementation of activity monitoring devices under commercial conditions. Detection of behavioral changes associated with estrus and parturition through the use of activity monitors will continue to drive further acceptance and use of these devices commercially, as these are important physiological events with significant economic and production impacts. Development of algorithms and analyses applied to activity monitor-based data as a measure of well-being and for the detection of disease are areas of active research in all sectors of the cattle industry.

Cows demonstrate altered lying behavior during clinical mastitis, as demonstrated under experimental and naturally occurring conditions. Based on video recording of behavior, cows exhibited a decrease in lying time for 12 hours following infusion of *E. coli* LPS into a single quarter, as reported by Zimov et al. (2011).[449] Siivonen et al. (2011) report significant decreases in lying time and time spent lying on the side of the affected quarter for 20 hours post-LPS infusion ($P < 0.07$), as well as an increase in stepping behavior ($P = 0.02$).[450] In Fogsgaard et al. (2012), cattle demonstrated similar changes following intramammary inoculation of *E. coli*, with a reduction in lying time and an increase in time spent standing idly for 24 hours post-inoculation.[451] Altered behavioral activity corresponded to peak rectal temperatures, evidence of

local inflammation, and increases in acute phase reactants.[449] Using activity data loggers, Cyples et al. (2012) further characterized the changes in lying behavior following intramammary LPS infusion.[452] Cows exhibited significant decreases in total lying time (73.3 minutes, $P = 0.005$), particularly during 4 to 7 h post-infusion, whereas no alterations in the proportion of time spent lying on the side of the affected quarter, number of lying bouts, or lying bout duration were found.[452] In de Boyer des Roches et al. (2017), sequential phases of experimental *E.coli* mastitis were distinguishable based on changes in behaviors and pathophysiological markers of an APR.[453] Behavioral changes preceded increases in APP and rumen temperature and returned to baseline prior to resolution of the on-going APR.[453] Cows were less attentive toward their surroundings, had lower head carriage, and changed posture (standing/lying) less often during the 8 h post-inoculation compared to pre-inoculation baseline.[453] Behavioral changes associated with naturally acquired mastitis are similar to experimental findings. In Medrano-Galarza et al. (2012), cows with mild mastitis spent significantly less time lying down and had a greater percentage of laterality behavior when lying (i.e. demonstrated a left or right side preference).[454] Unlike Siivonen et al. (2011), no differences in daily step counts were observed between healthy and mastitic cows.[454] Likely owing to the mild nature of clinical disease, no significant differences in weight distribution, stance, or stepping and kicks during the milking process were observed between healthy and mastitic cows; however, cows did tend to demonstrate more restless behavior during milking for 3 days after mastitis detection.[454] Fogsgaard et al. (2015) found several behavioral changes in mastitic cows, which persisted for at least 10 h after initiation of treatment.[455] As in Medrano-Galarza et al. (2012), mastitic cows demonstrated significant behavioral differences compared to healthy cohorts, including reduced lying time, increased lying bout frequency, and increased daily step counts, as well as altered behavior

during the milking process, with increased leg lifting frequency and kicks during milking in the AMS.[455]

In addition to changes in feeding and rumination behaviors as markers of health, the potential also exists for identifying cows at risk for illness during the transition period based on activity logger data. Huzzey et al. (2005) have characterized changes in eating, drinking, and behavioral activity of healthy free-stalled dairy cows during the transition period.[442] Daily standing time increased by 2 h during the calving period compared to the pre-calving period and 1 h longer than the post-calving period, as well as an 80% increase in the number of standing bouts during the calving period, reflecting increased activity around parturition.[442] Characterization of behavior in subclinical hypocalcemic dairy cows, Jawor et al. (2012) found these cows to stand 2.6 h longer during the 24 h prepartum, whereas they spent 2.7 h less time standing 24 h post-partum.[456] Itle et al. (2015) found that post-partum cows with clinical ketosis spent less time lying and increased time standing in the week before calving, whereas no differences in lying time were evident post-calving.[457] Kaufman et al. (2016) found no differences in lying time, lying bout frequency, or bout length in different health categories of primiparous cows during the transition period, whereas subclinically ketotic multiparous cows demonstrated increased lying time post-partum compared to healthy cohorts.[458] An increase of one standard deviation (131 min) above the mean daily lying time (601 min/d) was associated with 1.8-times increased odds of hyperketonemia associated with another postpartum disease during the first week post-partum.[458] Edwards and Tozer (2004) report identification of cows that developed left displaced abomasum, ketosis, and general digestive disorders, 5 to 6 d earlier than clinical examination, based on changes in daily walking activity and milk yield.[459] Liboreiro et al. (2015) also found reductions in postpartum activity in cows with subclinical ketosis, retained

fetal membranes, and metritis.[460] Similarly, Sepulveda-Varas et al. (2014) found differences in postpartum lying time differentiated healthy and clinically ill dairy cows under grazing conditions.[461] Primiparous cows that developed more than one clinical disease, excluding lameness, spent more time lying, and tended to have longer lying bouts postpartum compared to healthy cows, whereas no differences in lying time was seen in multiparous cows.[461]

Use of activity data loggers for behavior monitoring has demonstrated the ability to detect other important diseases in beef cattle, under experimental and naturally occurring disease states. In an experimental model of clinical *Mannheimia hemolytica* pneumonia, calves spent significantly more time lying, less time standing, and took fewer steps, as determined by accelerometer and pedometer quantification, compared to baseline activity.[462] Subtle sickness behaviors following BVDV challenge were demonstrable through the use of accelerometers in a mild BVDV infection model by Bayne et al. (2016), wherein BVDV-infected calves spent less time standing during the period of viremia, however, the ability to distinguish infected and non-infected calves using accelerometer-based data was limited.[463] Using ear-tag sensor triangulation technology, White et al. (2012) demonstrated significant reductions in activity level, including proximity to feed bunks, and less distance traveled in calves following intranasal inoculation of *Mycoplasma bovis*.[411] Recently, an ear-tag accelerometer has been evaluated for accuracy of activity monitoring of feedlot cattle under commercial conditions.[410] In Smith et al. (2015), ear-tag accelerometer activity data allowed the identification of moribund steers in central Kentucky.[464] Mean hourly activity counts of sick steers were approximately 25% lower than the activity counts of healthy steers for 16 of 24 hours per day.[464] Following treatment, activity counts of recovered steers returned to values similar to healthy cohorts.[464] In addition, accelerometers and other activity monitors have been used for lameness detection. Given the

complexity of kinetic and kinematic gait evaluation in the bovid, the use of activity monitors will be discussed in the last section of this literature review in the context of objective characterization of lameness in cattle.

Activity monitor behavioral data demonstrates exceptional usefulness in both research and commercial production settings, as the data generated serves multiple purposes. Accelerometers have demonstrated to be a practical and reliable tool for estrus detection and reproductive management. Continued investment in these technologies on commercial operations will in large part be based on this factor. Continued research needed to further utilize accelerometer based data include the critical assessment of management and husbandry practices and resultant improvements in cow-comfort and well-being. As well, the development of algorithms to detect behavioral changes associated with morbidity, thereby improving the timely detection of disease, monitoring treatment responses, and the overall health and productivity of cattle at an individualized level.

Lameness is one of the most costly health problem of dairy cattle and represents a major welfare concern across both the beef and dairy industries.[465,466] The incidence of lameness in dairy cattle varies between herds, but can range from 30 to 55% dependent on geography and management factors.[467] The estimated incidence in the feedlot sector is considerably less at <10%, but the issue in fattening and market-ready cattle has received limited attention, and the overall impact and prevalence of lameness conditions are likely underestimated.[468] There are multiple locomotion scoring systems used to assess lameness in dairy and finished cattle, varying in their respective complexity of parameters assessed.[469-473] As with BRD detection and subjective CIS, gait assessment in cattle demonstrates marked variability in accurate detection and agreement between observers.[474,475] To overcome the limitations of subjective clinical

assessment, research groups have used different technologies to objectively and quantitatively measure posture and gait. Three broad categories of approaches used include: kinetic, kinematic, and behavioral indices.

Detailed soundness evaluations for pre-purchase or diagnostic work-up for lameness are common in equine performance practice. Similar to limitations observed in cattle, subjective evaluation also lacks agreement for designation and quantification of mild- to moderate-degree lameness in horses.[476,477] Objective gait analyses enable the quantification of spatial and temporal variables of locomotion and static postural behaviors.[478] Kinetics is the study of forces involved in motion, whereas kinematics is the study of motion as it pertains to changes in body position over time.[478] Ground reaction forces (GRF) exerted on the hoof (or claw) by the ground are present during standing or walking. The vertical component of the ground reaction force is distributed over the contact area of the hoof (or claw) of a limb, dependent on the shape of the hoof (or claw) as well as its placement.[478] In turn, the vertical force distribution between lateral and medial claws and over the contact area of each claw determines the degree of local compression of the horn and underlying tissues. From a kinematic perspective, the step cycle of a limb during walking can be broken down into a weight-bearing (stance) phase and a non-weight bearing (swing) phase.[478] Several technologies for the objective quantification of lameness, extensively used in equine, have been increasingly use in livestock species. These include video-based and sensor-based computerized kinematic motion analyses systems and accelerometers, as well as GRF systems which may involve pressure-sensitive walkways, stationary force plates, and force-measuring treadmills.

Early use of force-plate analysis include the description by Scott (1987, 1989).[479] Using a commercial force plate system, vertical and horizontal ground reaction force-time curves were

generated in a group of seven cows. Importantly, this early work highlighted the variability in GRF changes demonstrated by cows with similar lameness severity and lesions. The majority of contemporary kinetic studies measure either the force exerted on the ground by the hoof as the cow walks on two parallel force plates, or measuring changes in weight distribution while the cow stands stationary on a platform comprised of four independent weight recording cells. Typically, most GRF systems are limited to variables captured in one or two dimensions, with limited research using more advanced, 3-dimensional systems.

Using pressure distribution and force plate systems, Van der Tol et al. (2003) reported the ground reaction forces, including the magnitude and distribution of pressures, exerted on the contact areas of forelimb and hind limb claws in healthy dairy cattle during the stance phase of walking.[480] Inclusion of five stance phase variables heel strike, maximum braking, midstance, maximum propulsion, and push off were utilized to determine pressure and contact area of individual claws and each foot. Differences were observed between the forelimb and the hind limb for the force and pressure distribution and their change over time during walking.[480] The maximum vertical ground reaction force on the forelimb was greater than the hind limb (3324 versus 2444 N, respectively, $P < 0.05$).[480] During walking, force was relatively equally distributed in the lateral and medial claw of the forelimbs, whereas force distribution was unbalanced in the hind limb, with a significantly greater part of the vertical ground reaction force exerted on the lateral claw.[480] Results highlight the need for high sampling frequency and spatial resolution, as pressure distribution on the bovine claw at a walk are dependent on the period of stance phase, and thus time. Further, the interindividual variation was higher in the forelimbs compared to the hind limbs.[480]

Similarly, in the evaluation of healthy, non-lame cows, Chapinal et al. (2009) report the influence of milking (e.g. pre-milking versus post-milking) and pregnancy status on weight distribution in Holstein dairy cows.[481] Evaluation included the use of a four-cell weight platform, as well as video recordings for subjective gait scoring and determination of walking speed. Following milking, there was a significant decrease in weight applied to both front and back limbs, with a greater decrease in the hind limbs compared to the forelimbs.[481] Variability of weight over time and the asymmetry of weight between contralateral legs, as an estimate for weight shifting, was not influenced by milking.[481] A small decease in gait score occurred post-milking, with the greatest appreciable changes being the degree of hind limb abduction or adduction. Walking speed remained the same post-milking.[481] Similarly, post-calving cows had reductions in weight applied to both fore and hind limbs, with similar decreases observed in all limbs.[481] Late-gestation cows demonstrated greater variability of weight over time in both fore and hind limbs.[481] Subjective scores and walking speed did not significantly change after calving, although post-partum cows demonstrated increased back arch scores and decreased asymmetry scores.[481]

In cows standing stationary, use of weight shifting between legs has been utilized as a measure of restlessness and pain. Steps, without walking, are a clear form of weight shifting between limbs. The frequency of steps and weight shifting when cows stand without walking were found to be positively correlated in dairy cattle, standing either on rubber or concrete surfaces.[482] Pastell and Kujala (2007) report the weight distribution among legs as automatically collected by load cells during robotic milking and the use of probabilistic neural networking to generate a model for lameness detection.[483] Combining the weight distribution variables of step frequency, weight shifting, and asymmetry provided an accuracy of 86% in the detection of

lameness.[483] Chapinal and Tucker (2012) report optimized thresholds for the use of weight shifting, as assessed by a weight distribution platform, for step frequency during standing in adult dairy cows.[484] In contrast to the threshold of 20 kg used by Pastell and Kujala[483], the optimal thresholds for classification of a step were considerably higher (127 and 98 kg for front and rear legs, respectively).[484] Lame cows took more steps with the rear limbs per minute than nonlame cows, whereas no differences were observed in frequency of steps with the forelimbs.[484] For the prediction of hind limb lameness, the frequency of steps was similar to the standard deviation of weight applied (i.e. weight shifting; AUC = 0.71) and the leg weight ratio (i.e. asymmetry; AUC = 0.69, as previously reported[485]). This is similar to the AUC of 0.86 reported by Pastell and Kujala.[483] Furthermore, although step frequency while standing and standard deviation of weight applied to the legs (i.e. asymmetry) are measures in weight distribution and demonstrate similar accuracies for lameness detection, these parameters do not measure the same phenomena and the authors recommended utilization of both.[484]

Liu et al. (2011) evaluated the relationship between clinical assessment of lameness to vertical kinetic (force plate) parameters in cows with unilateral lameness.[486] Determination of pain-related indices were based on withdrawal response using an algometer probe and a Dillon force gauge, which were incorporated into a clinical locomotion score. In agreement with previous reports, measures of kinetic limb loading decreased with increasing lameness scores.[486] These differences in limb loading led to significant asymmetries between the hind limbs in cows with unilateral lameness.[486] Lame cows demonstrated compensatory limb loading, with changes in stance duration in affected and non-affected limbs.[486] However, lameness with similar lesion distribution, lesion severity, locomotion score, and pain reactions produced more than one effect

on vertical kinetic limb variables, with variability in compensatory mechanisms demonstrated between cows.[486]

Pastell et al. (2010) compared subjective numerical lameness scores and leg weight ratios (i.e. asymmetry between paired hind limbs or forelimbs) and the detection of lameness due to different etiologies.[487] Significant correlation between leg weight ratios and lameness scores were found for non-infectious lameness, e.g. sole ulcers ($r = 0.79$) but not for lameness due to digital dermatitis.[487] Surprisingly, no correlation between the sole ulcers or lameness and the variability of the weight placed on each leg over time (i.e. shifting of weight between legs) was present, unlike previous findings of the same group.[483] However, use of leg weight ratios could differentiate lame cows caused by non-infectious etiologies from sound cows with no sole lesions (AUC = 0.87), with increasing accuracy of this kinetic parameter with increasing severity of gait scores.[487] However, large variance of leg weight ratios was observed in sound cows, which presents a challenge of detecting mild lameness using a single kinetic parameter in isolation. In addition, not all lame cows were detectable with use of leg weight ratios alone, as some lameness was more marked during walking compared to when standing.[487]

Comparison of subjective gait scores and GRF is reported by Ghotoorlar et al. (2012), utilizing a four-cell ground force plate in standing dairy cows.[488] Grouping cows based on lameness scores (1 – 5), GRF measures demonstrated good sensitivity and specificity for lameness grades (1 – 4), with greater than 72% sensitivity and specificity in these groups.[488] Assessment of grade 5 lameness was limited in sample size and demonstrated a poor sensitivity of 50%. A great sensitivity (94%), specificity (100%), positive (100%) and negative (98%) predictive values in the detection of sound cows make the system suitable for screening programs.[488] The system requires cows to stand still for approximately 6 minutes for data

collection, and thus would be most appropriate for use in the milking parlor or robotic milker.[488] In contrast, poor sensitivity and performance of a commercial system was reported by Bicalho et al. (2007), who evaluated the associations between visual locomotion scoring (VLS) and a commercial GRF automated locomotion system (Stepmetrix locomotion scoring, BouMatic, Madison, WI).[489] In 459 cows, sole ulcers were the most prevalent hoof lesions (53%), followed by digital dermatitis, white line abscessation, toe and heel ulcers, double soles, foot rot, and other diseases. At the manufacturers' recommended cut-offs, the automated GRF system demonstrated a sensitivity and specificity of 22.2% and 93.8%, respectively for the detection of lameness, with visual locomotion scoring performing better than the commercial system.[489]

The impact of hoof trimming on gait score, activity indices, walking speed, and weight distribution parameters, as reported by Chapinal et al. (2010), demonstrated use of objective measures to continuously monitor animals to detect onset or recovery from lameness.[490] Before hoof trimming, lame cows (based on subjective gait scores) demonstrated uneven rear weight distribution (asymmetry), more weight shifting when standing, and spent more time lying down than sound cows.[490] No differences in walking speed between lame and sound cows were observed before hoof trimming.[490] Importantly, hoof trimming had an impact on both lame and sound cows, with a deterioration in gait scores, a reduction in walking speed and an increase in lying time.[490] Lame cows showed an increase in weight distribution asymmetry between the hind limbs following hoof trimming not observed in the sound cows.[490] The change in time spent lying after hoof trimming was correlated with the change in gait score and change in the leg weight ratio, i.e. asymmetry between hind limbs.[490] Similarly, Chapinal et al. (2010) objectively evaluated the impact of pain mitigation (i.e. non-steroidal anti-inflammatory administration, i.e. ketoprofen) and hoof trimming in dairy cows using objective measures of weight distribution and

daily accelerometer activity data.[491] Gait scores were positively correlated with standard deviation of weight distribution of hind limbs (r = 0.32, *P* = 0.01) and negatively correlated with rear leg weight ratio (r = -0.52, p = 0.002) and step count (r = -0.43, *P* < 0.001).[491] As a measure of asymmetry between contralateral limbs, the leg weight ratio was the best measure for identifying lame cows.[491] Use of activity monitoring indices showed limited correlations with gait scores and, although a significant correlation was found between lameness score and step counts, most of the steps were associated with milking and therefore the influence of milking frequency and the distance from pen to milking parlor should be considered.[491] Use of analgesia had little effect on subjective lameness scores, whereas the impact on weight distribution measures was not clear. A tendency for a decrease in limb shifting in cows receiving an NSAID following hoof trimming was observed, but the magnitude was small (less than 5kg).[491]

As discussed above, there are disagreements as to the best variable or variables to measure when using kinetic data to detect lameness. Whereas asymmetry was found to be most accurate in Pastell et al. (2010), both asymmetry in limb weighting and shifting between limbs were good measures, but the latter was more accurate in Chapinal et al. (2010).[487,491] The inherent variability present between different types or forms of lameness will likely mean different parameters are better suited for the detection of different conditions. For example, a lameness that does not result in different limb loading at rest but alters GRF exerted by different limbs at the walk would be best detected using a pressure mat capable of measuring kinetic parameters during motion, as opposed to a stationary plate capturing GRF while standing. A stationary plate capturing GRF would be more suitable for a lameness not affected by motion, with unequal weight distribution patterns at rest. Correspondingly, shifting within the same limb will not necessarily result in a positive finding when 1-dimensional load cells are utilized to

capture GRF, as these have limited ability to discriminate between the claws of the same limb. Use of more advanced systems, which incorporate data in three-dimensions (not limited to the vertical dimension), will likely lead to more accurate lameness detection, as detecting load shifts between claws would be possible. For example, Dunthorn et al. (2015) report a 52% sensitivity and 85% specificity for lameness detection using a force plate system restricted to the vertical dimension.[492] Modification of the system to measure ground reaction forces in three dimensions improved the sensitivity and specificity for lameness detection to greater than 90%.[492] Thorup et al. (2014) report differences in ground force reaction curves of sound and lame cows and the impact of hoof trimming, using a system comprised of two parallel, 3-D strain gauge force plates, over which cows repeatedly walked.[493] Vertical, longitudinal, and mediolateral GRFs were quantified over time, as well as walking speed measured. Symmetry parameters for each of the three force directions were calculated to compare the entire stance phase curves of the paired left and right legs.[493] Walking speed was significantly greater in sound cows compared to lame cows ($P < 0.001$).[493] Following trimming, all 3-D symmetries were significantly improved, however short-lived, as the effect was not present for mediolateral and longitudinal direction by day 7 post-trimming.[493] The presence of lameness significantly worsened vertical and mediolateral symmetries compared to sound cows.[493]

Maertens et al. (2011) described the automated Gaitwise system, which incorporates kinematic and kinetic variables using a pressure sensitive walkway (ILVO, Merelbeke, Belgium)[u].[494] In relation to subjective gait scoring, the system had an overall sensitivity of 76 – 90% and specificity of 86 – 100%. Important parameters with discriminatory capability included: asymmetry in step length, stance time, step time, and step width, whereas asymmetry in relative force of limb surprisingly did not add to the correlation. Clinically, the device was reported to be

simple and relatively inexpensive to implement on farm.[494] However, further improvements needed to ease data capture and use include the ability to link locomotion data with RFID information in real-time. Van Nuffel et al. (2016) evaluated cow-related and environmental factors that affect cow locomotion and the detection of lameness using the Gaitwise system[u].[495] Environmental effects, such as wet surfaces, dark environment, as well as the cow-related factors of age, production level, lactation and gestational stage impacted gait and should be taken into account, as an improvement in the number of false alerts were found when these variables were accounted for with this system.[495]

Assessment of biometric tools in kinetic gait analysis of young calves is currently limited. Information on baseline biometric variables, including stance time, stride velocity, pressure distribution, impulse and maximum forces using a floor mat-based pressure/force measurement system were reported for clinically normal calves.[496] Large variability within and among individual calves was present, especially as it pertained to stance time and stride velocity.[496] Conversely, impulse and maximum force was less varied and likely these two variables might be clinically useful for the detection of lameness in young calves.[496] The authors suggested the establishment of a baseline of biometric values for each calf and monitoring changes over time, rather than comparison of a single measurement with a reference range.[496] Evaluation of kinetic gait parameters and the efficacy of several cyclooxygenase inhibitors in an amphotericin B-induced synovitis-arthritis calf model have been reported, using similar biometric technology.[497-499] Overall, alteration in kinetic parameters were appreciable following synovitis-arthritis induction.[497-499] As demonstrated either by kinetic parameters returning towards baseline more readily, or a lesser change in magnitude of kinetic parameters compared to control animals, amelioration of discomfort and lameness, to some degree, was observed in calves administered

flunixin meglumine[498], or meloxicam with or without gabapentin[499], whereas administration of sodium salicylate had no overall difference on kinetic parameters.[497] Accelerometer activity indices also indicated differences in behavioral activity with NSAID administration, wherein treated calves spent less time in recumbency[498] or had increased step counts compared to controls.[499]

The earliest description of quantitative kinematic gait analysis in dairy cattle by Herlin and Drevemo (1997) utilized high-speed cinematography of hand-walked cows and processing of video data via commercial motion analysis system.[500] This work characterized walking speed, stride duration and the percentage of time of each phase of the stride cycle, as well as various joint angles, in 17 Swedish Friesian cows housed under different management conditions.[500] Other early efforts using computer-aided kinematic techniques, commonly used in horses, include the work of Flower et al. (2005).[501] Video recording of dairy cows walking were performed on seven consecutive days as cows exited the milking parlor and traveled down a concrete test alley. Cows were fitted with reflective markers above the fetlock joint of each leg to ease visualization and at least two consecutive strides per cow were recorded. Kinematic measures calculated were spatial parameters (i.e. stride length and maximum stride height) and temporal parameters (i.e. stride, stance, and swing duration and hoof speed). Clinical assessment of hooves and morphometric measures (e.g. body mass, height at withers and tailbone, and back length) were also assessed.[501] Cows with sole ulcers had significantly shorter stride lengths, longer stride duration, and had lower maximum stride height compared to healthy cows ($P < 0.05$).[501] Lame cows also had slower average hoof speed ($P < 0.001$).[501] The proportions of the total stride duration made up by the stance and swing phases also differed between lame and sound cows, with the percentage of triple support during the gait cycle (time when animal

supported by 3 legs) more than doubled for cows with sole ulcers compared to healthy cows (42 versus 18%, $P < 0.001$).[501] Subsequently, the same group demonstrated changes in kinematic variables following milking, in both sound and lame cows with sole ulcers.[502] Following milking, all cows had significantly longer stride length, higher maximum stride height, shorter stride duration, shorter periods of triple support, and faster walking speeds ($P < 0.05$).[502] Significant differences in kinematic variables were even more apparent between lame and sound cattle post-milking.[502] Results suggest that kinematic evaluation of gait in dairy cows should be performed after milking to limit the influence of udder distention on gait variables.[502] Flooring substrate, e.g. concrete compared to composite rubber flooring, alters kinematic variables in both sound and lame dairy cows.[503] An additional spatial parameter not previously calculated included stride overlap (e.g. horizontal distance between front hoof strike and subsequent ipsilateral rear hoof strike).[503] On composite rubber flooring, cows overall had longer and higher strides, greater overlap between front and hind hooves, longer swing durations, less time in triple support, and a faster walking speed.[503] The correlations between gait scores (tested on concrete, 1 - 5) and differences in kinematic variables between concrete and composite rubber flooring were greatest for stride length, swing duration, and triple support, whereas non-significant correlations were found for stride height, stride and stance duration, and walking speed.[503] Interestingly, the subjective gait score reflected how the cow responded to the flooring surface – cows with the more severe lameness scores benefited the most from a softer, higher-friction surface.[503]

Blackie et al. (2013) assessed the impact of different foot lesions on the various kinematic gait phase parameters in lame dairy cows.[504] Markers at the center of rotation were attached to the fore and hind limbs, as well as along the spinous processes. Gait cycles were recorded using a digital video camera and motion analysis software used for analyses.[504] In

addition to commonly reported gait cycle parameters, joint flexion angles and heights, as well as height of spinal processes, withers, and head position were determined. Similar to the findings of Flower et al. (2005), shorter stride lengths and tracking distances (P = 0.032 and 0.06, respectively) were observed with increasing locomotion scores.[504] A weak tendency of longer stride duration was also observed with increasing severity of lameness.[504] Locomotion scores had no association with joint heights and a limited association with joint flexion (e.g. range of motion).[504] Spine marker height on thoracic and lumbar spinal processes did not differ between locomotion scores, however, subtle changes to spine length and posture were apparent in cows with sole ulcers.[504] The presence of sole ulcers had the greatest impact on differences in gait parameters, as compared to cows with sole hemorrhages or healthy cows.[504]

Use of treadmills for kinematic gait analysis offer the advantage of standardization. Due to multiple factors, including availability, cost, and animal temperament, use of treadmills in cattle is limited, especially when compared to research carried out in horses. Meyer et al. (2007) report the ground contact patterns of the feet during the first half of the stance phase in non-lactating heifers, using high-speed cinematography and a treadmill.[505] Peak vertical ground reaction forces were also measured in all four limbs, however the system could not distinguish between the lateral and medial claw. The stance and swing duration did not differ between fore and hind limbs. Peak vertical GRF was significantly higher in the forelimbs compared to the hind limbs. Both forelimbs and hind limbs demonstrated laterality of claw contact, with the lateral claw contacting the ground prior to the medial claw.[505] Laterality was more pronounced in hind limbs, e.g. longer lag time before both claws made ground contact.[505] Similar overall gait patterns and claw-ground contact patterns in lactating dairy cows were found by Schmid et al. (2009), using a treadmill and high-speed cinematography approach.[506] Similar to heifers, the

lateral claw made contact with the ground before the medial claw in both fore and hind limbs in cows.[506] Differences in gait pattern between cows and heifers include a greater step width in cows, with the authors speculating that this may lead to increased shear and horizontal ground reaction forces placed on the lateral claw.[506] In addition, the time lag between ground contact time between claws was longer in cows (35.5 ms)[506] when compared to heifers (24.0 ms)[505]; and, laterality was slightly longer in the forelimb of cows compared to the hind limb of heifers.[506]

Accelerometers have been most useful for detecting standing and lying behavior, whereas refinement of data analyses for the characterization of detailed kinematic parameters during walking are still needed, including the optimization of sampling frequency. High sampling rates are more suitable for precise gait analyses, but such frequencies overwhelm the battery life and data storage capabilities of most commercial activity monitors. Changes in trunk and limb acceleration as a measure of gait alteration has been evaluated using 3-dimensional accelerometer data in adult dairy cattle. Using accelerometers strapped to the lateral aspect of each limb, just proximal to the fetlock joint, and a single accelerometer on the back mid-line, Chapinal et al. (2011) acquired acceleration data at the walk in lactating dairy cows on different flooring surfaces.[507] Comparisons between subjective gait scores, kinematic variables, including the mean and variance of acceleration of paired front and rear legs and back, as well as walking speed were performed. The asymmetry of variance of acceleration in the front and rear legs was positively correlated to gait scores and visually assessed asymmetry of steps.[507] In other words, cows with high gait scores (i.e. more severe lameness) had greater asymmetry in the variance of acceleration in both front and rear legs. This agrees with the findings of Pastell et al. (2010), although only rear limb asymmetry of variance of acceleration were found.[487] Walking speed was positively correlated with the mean acceleration and variance of acceleration of the back and

each leg, whereas it was negatively correlated with the asymmetry of variance of the rear legs and the asymmetry of the steps.[507] However, no correlation was observed between gait score and mean walking speed. Differences in acceleration measures were identified between concrete and rubber flooring.[507] Tanida et al. (2011) describe gait characteristics at the walk in 6 Holstein cows using a 3-D accelerometer attached to the metacarpal region. Simultaneous video recording of each cow was performed, with markers affixed to the carpus, hock, and fetlocks of the right fore and hind limb, with accelerometer data synchronized with video images.[508] This approach allowed the identification of individual steps, each comprised of two discernable phases (i.e. acceleration and non-acceleration) using Lissajous figures.[508] Description of whole body acceleration using the same accelerometer model, affixed to the thoracolumbar region, was also evaluated in 17 Holstein cows. Hoof trimming significantly decreased both the range of vertical movement of the joints in both the fore and hind limbs, as well as the variance of lateral and forward acceleration of the spine sensor.[508] This suggested improved smoothness and rhythmicity of gait following hoof trimming. Interestingly, the trajectory pattern of the leg movements could be reproduced by measures of vertical acceleration of the dorsally attached accelerometer.[508]

Alsaaod et al. (2015) developed algorithms to quantify activity indices and gait parameters utilizing 3-D accelerometer data in loose-housed dairy cows, using video-recording as the gold standard comparison.[509] Of interest from a kinematic perspective, algorithms were developed to characterize stride length and stride duration. Strong correlations were found for the activity indices of lying, standing, and walking (time and bouts, as well as step frequency) between video and accelerometer data.[509] The relative measure of error (RME) for the parameters stride duration and length were greater but reasonably strong correlates were

demonstrable. Correlation between video recording and accelerometer data for stride duration and stride length were 0.75 and 0.81, respectively.[509] The corresponding mean RME for stride duration and stride length were 6.65 and 11.92%, respectively.[509] In continuation, Alsaaod et al. (2017) further refined accelerometer data analyses for kinematic gait cycle assessment in cattle at the walk. This included comparison of acceleration between the lateral hind claw and metatarsus of the same limb, in sound and lame cattle.[510] Devices used included a high-speed video camera, two high-frequency accelerometers, as well as inertial measurement units (IMU), designed for use in humans. The IMU gait analysis system included a 3-D accelerometer, a 3-D gyroscope, and a magnetometer. Only the accelerometer data from the IMU were utilized, which were synchronized with cinematographic data. Kinematic data were transformed into a single vector magnitude (pedogram) and were compared to cinematographic data.[510] See[510] for detailed account of pedogram parameters. Overall, measurement of gait cycle variables at the level of the metatarsus, using a single accelerometer (with a high sampling rate), was adequate for the indirect characterization of acceleration of the claw.[510] Differences in all kinematic variables of the gait cycle, with the exception of gait cycle duration, were significantly different between sound and lame cows.[510] Measurement of gait cycle variables using two accelerometers, on respective metatarsi, differentiated between sound and lame cows with unilateral lesions of the hind feet.[510] Use of two accelerometers, placed on each metatarsus, may be a useful tool to accurately describe the different gait cycle variables as well as detect unilateral hind limb lameness. Next, Alsaaod et al. (2017) reported the validation of a semi-automated software system (Cow-Gait-Analyzer) in order to extract the relevant gait events from accelerometer data.[511] Low RME for the parameters: gait cycle duration, stance phases, foot load, and toe-off

peaks were found.[511] Thus, the semi-automated software tool for high-frequency accelerometer data analyses was found suitable for detailed kinematic gait pattern characterization in cows.[511]

Poursaberi et al. (2010) describe the use of 2-D imaging processing techniques and the development of an automatic real-time algorithm to quantify back posture measurements, for the detection of lameness in dairy cattle. A correct lameness classification rate of 96% was found.[512] Likewise utilizing computer 2-D vision techniques for the extraction of back posture parameters, Viazzi et al. (2013) assessed individual cow variation in body movement patterns (BMP) to generate individualized thresholds for lameness detection. Individualized values were compared to population-based threshold modeling.[513] Using a population-based threshold had an overall classification accuracy of 76%, a true positive rate of 83%, and a false positive rate of 22%, when 3 classifications of locomotion status were used.[513] Overall accuracy was improved to 85% with binary classification (i.e. non-lame and lame). When an individualized threshold was used for each cow, the overall accuracy and true positive rate increased to 91%, and the false positive rate was decreased to 6%.[513] Furthermore, classification rate was high for all 3 lameness classes. Generation of individualized BMP and thresholds seems a reasonable approach to overcome individual variability for lameness detection, when using image analysis systems.[513] Modifications of camera positioning and the inclusion of 3-D analysis were subsequently carried out by Viazzi et al. (2014).[514] Both 2-D and 3-D algorithms demonstrated similar accuracy of 90% for the classification of lameness.[514] The 3-D top-view algorithm approach offered the advantage of fully automated image segmentation and analysis in real-time, as well as the capability of assessing cows walking side by side in tandem.[514] Hertem et al. (2014) further optimized the classification performance of the previously described algorithm[515], by including consecutive measurements of the same animal.[515] The model demonstrated a reasonable accuracy

of 60.2% using four consecutive measurements when compared to a 5-point gait score.[515] Accuracy was improved to 81% when a binary classification was used[515], however this was less than the 90% previously reported for the same system.[514]

Characterization of gait patterns in cattle has been enhanced with the application of kinetic and kinematic technologies. Although most of these technologies are currently limited to research settings, implementation of such devices will likely continue to grow commercially as the cost and capabilities of these devices improves. With an increasing interest in AMS, capture of GRF by milking platforms equipped with load cells, is a very feasible and practical way kinetic parameters can be measured daily, with the generation of alerts as to the presence of lameness in individual cows. Quantification of both kinetic and kinematic data by pressure sensitive walkway systems, such as Gaitwise, also offers a real world, practical approach to lameness detection. Although improvements are needed in the accuracy and implementation of these systems on commercial operations, they are straightforward in concept. Most current kinematic technologies require multiple devices and a level of expertise for data interpretation that limits their application to the research setting. Defining which parameters are best suited to fully capture gait patterns, and development of algorithms which accurately detect lameness, needs further attention. However, with continued improvement in battery life and data memory storage capabilities, accelerometers capable of high frequency data acquisition in the future may be able to quantify kinematic gait parameters under field conditions in real-time. As well, niche sectors of the cattle industry, such as performance animals (e.g. bucking stock), would benefit from the development of a kinematic-based sensor system for gait analysis for characterization of performance limiting lameness - akin to the lameness detector systems available in equine performance horse medicine (e.g. Equinosis™ lameness locator®)[v]. At the present time, use of

remote technologies to detect lameness largely utilizes accelerometer based data, using activity monitors implemented for estrus detection as well as overall health.

Activity monitor data have proven useful for detecting lameness based on changes in standing and lying time, lying bout frequency and duration, as well as intensity of motion indexes (proprietary algorithms) and step counts. Incorporation of multiple modalities, such as rumination activity, bunk attendance systems, or kinetic/kinematic variables, along with production parameters (e.g. milk weight), improves the accuracy of these systems to detect lameness.

Mazrier et al. (2006) evaluated whether a reduction in pedometer activity based on individualized baseline activity would be capable of detecting lameness in free-stalled dairy cows under field conditions.[516] An arbitrary threshold for reduced pedometer activity was set at 5% and baseline activity defined as the average daily activity for 10 d before lameness detection.[516] Of the cows demonstrating a 5% decrease in pedometer activity, 45.7% developed clinical lameness within 7 – 10 d, whereas the other 54.3% did not develop evidence of lameness or other disease (representing false positives).[516] For cows with both decreases in pedometer activity and clinical lameness, 92% had decreases in pedometer activity greater than 15%.[516] However, the insensitivity of using step counts alone was highlighted by 44.7% of clinical lameness diagnoses on farm represented false negatives, as these cows did not demonstrate reductions in pedometer activity.[516]

Based on activity data from four commercial dairy farms, Thorup et al. (2015) demonstrated significant differences between lame and non-lame cows that paralleled increases in lameness severity.[517] Based on single activity variables, total acceleration while walking (motion index) and walking duration allowed the detection of mild lameness, whereas changes in

lying duration only became evident at higher lameness scores.[517] In addition to moderately-severely lame cows increasing lying duration by 40 min/d, they also demonstrated increased restlessness during standing based on the motion index parameter.[517] Importantly, a large amount of individual variation in activity parameters was present, especially in severely lame cows[517], similar to the findings of Alsaaod et al. (2012).[518] Collectively, this reinforces the suggestion that detecting derivations in activity may be better served by individualized baseline activity. Likewise, Yunta et al. (2012) evaluated accelerometer-based data from 10 free-stall commercial dairy herds under similar management and feeding regimes using a mixed-effect model.[519] Lame cows demonstrated increases in lying bout duration, whereas total daily lying time, number of lying bouts, or laterality were not affected by lameness.[519] Interestingly, a relationship between the time to standing up and lying down relative to feed delivery was different between lame and non-lame cows, with lame cows standing 13 min later ($P < 0.05$) and laying down 19 min earlier ($P < 0.05$) after feed delivery.[519]

Westin et al. (2016) report similar changes in lying behavior in lame cows managed with automated milking system (AMS).[520] On 36 United States and Canadian dairies utilizing AMS and activity-monitors, lame cows in general spent 0.6 h/d increase in lying time, in fewer, longer lying bouts compared to sound cows, although large variations in lying time between individual cows was noted.[520] Importantly, increased lying time was also associated with increased parity, later stage of lactation, and higher body condition score.[520] Alsaaod et al. (2012) report use of accelerometer and pedometer activity data with machine learning data analysis methods for lameness detection.[518] Important findings included small differences in activity between lame and sound cows, with use of absolute values and thresholds insufficient for lameness prediction.[518] In addition, the differences between individual cows was very high compared to

the difference between the means of lame and non-lame days of the same cow, such that changes in step frequency due to lameness were not predictable between cows – e.g. daily step count increased for 6 cows, whereas it decreased in 5 cows.[518] Rather, deviation from normal behavior yielded better accuracy than absolute values.[518] Similarly, De Mol et al. (2013) utilized the usefulness of day-to-day behavior variation as a means to detect derivation from normality for the detection of lameness.[521] Based on behavioral activity indices, concentrate intake, and milk yield, a dynamic linear model was generated for the detection of lameness in loose-housed dairy cows, managed using an AMS.[521] The overall sensitivity was 85.5% and specificity of 88.8%.[521]

Chapinal et al. (2009) evaluated changes in gait scores, walking speed, and lying behavior associated with hoof pathologies in lactating Holstein cows.[522] Numerical rating scores (NRS, i.e. a gait score based on 7 gait attributes) were correlated with head bob, tracking, joint flexion, asymmetric steps, and reluctance to bear weight; as well, these gait variables were correlated with one another. However, the reluctance to bear weight was the only variable found to be a predictor of sole ulcers in final stepwise modeling of the parameters ($R^2 = 0.11$, $P = 0.02$). A NRS threshold of 3.0 had sensitivity, specificity, and accuracy values of 85, 38, and 49%, respectively. Whereas a threshold of 3.5 had a sensitivity, specificity, and accuracy of 54, 70, and 66%, respectively. Changes in NRS were predictive of ulcer development at least 4 weeks before the ulcer was clinically visible, and increases in NRS were apparent for a minimum of 4 weeks after ulcer diagnosis in peripartum cows.[522] Walking speed was negatively correlated with NRS and gait attributes, however, walking speed did not differ across hoof health categories (e.g. normal, sole hemorrhages, digital dermatitis, and sole ulcers). Lying activity, as quantified using accelerometer-based data, demonstrated increased duration of lying bouts and resultant higher daily lying time in cows with sole ulcers compared to all other hoof health categories.[522]

Nechanitzky et al. (2015) evaluated the suitability of several automated measures to assess altered behavior in cows associated with unilateral hind limb lameness.[523] Measures included weight distribution, feeding behavior at night, heart rate activity, as well as standing and lying behavior at night. Pertaining to accelerometer activity, lame cows spent significantly more time lying and less time standing and walking ($P = 0.049$). The number of lying bouts did not differ between sound and lame cows, and thus lying bout duration contributed to the increase in lying time in lame cows. When comparing the different modalities, the weight platform data was superior to accelerometer-based data in distinguishing lameness.[523]

Van Hertem et al. (2013) developed a mathematic model to detect lameness based on existing sensor data on a commercial dairy farm.[524] All cows were fitted with a neck collar tag (HR-Tag, SCR Engineers, Ltd.)[l] used for automated heat detection, which quantified neck movements and rumination. Also included in the model was the performance variable milk yield and classification of health status was dichotomous, i.e. sound or lame. Milk yield 4 days before the diagnosis of lameness demonstrated the highest correlation of all variables. For activity data, sound cows had higher neck activity during the day compared to lame cows, resulting in a lower night to day ratio of neck activity. The 7-d average of the night period to day-period neck activity ratio was significantly lower for lame cows ($P < 0.001$). Seven variables were retained in the model, with a reported sensitivity, specificity, and accuracy of 89%, 85%, and 86%, respectively, for the detection of lameness.[524] Thus, exploitation of preexisting activity data used for estrus detection on-farm, combined with production data, was successful in the detection of lameness. Similarly, combining the monitoring modalities of accelerometer activity and noseband sensors, Beer et al. (2016) demonstrated the parameters of standing bouts and walking speed of greatest utility for lameness detection, with a model sensitivity and specificity of 90.2 and 91.7%,

respectively.[525] Rumination-related activity of lame cows included significant reductions in eating and rumination time, fewer chewing movements during eating and ruminating, and a decrease in the number of rumination boluses.[525] Locomotion activity changes associated with lameness included increased lying time and lying bout duration, decreased standing time, fewer standing and walking bouts, as well as slower, shorter strides and a slower walking speed.[525]

Similar to the detection of BRD and periparturient diseases in beef and dairy cattle, respectively, the use of accelerometer based activity data demonstrates the ability to detect lameness. The increasing use of activity monitors and other modalities on dairy farms will facilitate the continued improvement of algorithms for the detection of disease, including lameness, under commercial conditions. Further research efforts are needed in the various beef cattle sectors, as the majority of research evaluating remote monitoring technologies has taken place in housed dairy cows. Feedlot conditions are conducive to the implementation of remote technologies, as these animals are intensively managed and housed in confined pens. However, research is strikingly limited under extensive pasture conditions in both beef, and to a lesser extent dairy, as the placement and retention of monitors, as well as the timely retrieval of data can be challenging under such conditions. However, cow-calf and stocker/backgrounding operations represent a vital and large portion of the beef cattle sector. Exploration of practical and economically important implementation of remote monitoring technologies needs to be further explored in these sectors, since management of well-being and health impact the cattle industry at large.

Table 1. Acute phase proteins in cattle

Acute phase protein	Category	Concentration during Health	Concentration during Acute phase reaction	Reference
Haptoglobin (Hp)	Major	<0.1 g/l	1.62 ± 0.47 g/l (±SEM) 1.26 ± 0.66 mg/ml (±SD)	[116] [128]
		32.8 ± 20.0 (serum, ±SEM); 1.8 ± 0.4 (milk, ±SEM) μg/ml	317.7 ± 37.5 (serum, ±SEM); 152.2 ± 22.6 (milk, ±SEM)	[121]*
		<0.1 mg/ml	>1.25 mg/ml	[216]^
		1.17 ± 0.07 mg/dl (±SE)	21.71 ± 3.32 mg/dl (±SE)	[213]
		0.08 ± 0.03 g/L (±SD)	2.95 – 4.96 mg/dl	[184]$
		0.79 ± 0.06 g/l (serum); 0.061 ± 0.01 g/l (milk)	1.31 ± 0.52 g/L (±SD) 1.89 ± 0.16 g/l (serum); 0.53 ± 0.01 g/l (milk) subclinical mastitis 2.71 ± 0.21 g/l (serum); 0.99 ± 0.02 g/l (milk) clinical mastitis	[207]! [191]
Serum amyloid A (SAA)	Major	1.3 ±0.4 mg/l (±SEM)	115 ±37 mg/l (±SEM) 4000 - >15,000 μg/ml (range)	[136] [195]#
		<0.3 μg/ml	73.2 – 298.2 μg/ml (range)	[526]&
		8.89 ± 0.72 μg/ml (±SE)	22.19 ± 0.85 μg/ml (±SE)	[213]
		4.49 ± 0.57 μg/ml (±SD)	179.5 ± 67.7 μg/ml (±SD)	[207]!
		34.6 ± 3.92 mg/l	151.61 ± 17.6 mg/l (subclinical mastitis); 189.72 ± 29.3 mg/l (clinical mastitis)	[191]
Mammary serum amyloid A3 (MSAA3)	Major	<0.3 mg/l	23; 4.4 – 103 mg/l (median; range)	[137]
		267.5 ± 154.1 μg/ml (colostrum, ±SEM) and 2.63 ± 3.47 μg/ml (milk, ±SEM)		[142]
		9.7 ± 0.1 μg/ml	24.6 ± 5.1 μg/ml	[141]
		13.6 ± 1.62 mg/l	22.41 ± 4.6 mg/l (subclinical mastitis); 36.19 ± 3.9 mg/l (clinical mastitis)	[191]

Acute phase protein	Category	Concentration during Health	Concentration during Acute phase reaction	Reference
Alpha1-acid glycoprotein (AGP)	Moderate	0.2 – 0.45 g/l	1.1 ± 0.44 g/l (±SD) 377.1 ± 24.64 mg/l (±SE) 580.0 ± 91.17 mg/l (±SE)	[527] [186] [186]†
Lipopolysaccharide binding protein (LBP)	Moderate	1.7 ±0.3 g/l	11 ± 12 g/l (±SEM) 6.3 ± 0.72 mg/l (±SE) 13.5 ± 3.29 mg/l (±SE)	[155] [186] [186]†
Fibrinogen	Moderate	2.08; 1.58 – 2.94 g/l (median; range) <200mg/dl 1.40 ± 0.17 g/l (±SE) <6 g/l 206.0 ± 64.8 mg/dl (±SD) 2.2 ± 0.18 g/l (milk)	2.79; 2.13 – 5.0 g/l (median; range) >400mg/dl 3.97 ± 0.22 g/l (±SE) 7 – 15 g/l (range) 518.9 ± 117.4 mg/dl (±SD) 7.89 ± 0.81 g/l (subclinical mastitis); 8.73 ± 1.22 g/l (clinical mastitis)	[528] [216]^ [213] [177]@ [207]! [191]
Albumin	Negative	35 ± 1 g/l (±SEM)	34 ± 1 g/l (±SEM)	[102]

* 12-h post-LPS challenge

^ Day 3 post *Mannheimia hemolytica* vaccination

$ Range of concentrations of calves treated 1, 2, and 3-times for BRD during the first 21 DOF

! Traumatic reticuloperitonitis (n = 53) reported for acute phase reaction

† Presence of *Pasteurella multocida* on tracheobronchial lavage fluid (n = 12), total 84 calves with respiratory disease in Nikunen et al. (2007)

48-h measurement in increasing severity scores of mastitis

& Range of SAA in cows (n = 10) with chronic inflammation

@ Range of peak fibrinogen concentrations in calves (n = 6) inoculated with BVDV and Mannheimia hemolytica (estimated from graph in publication)

Table 2. Bovine acute phase protein profiles described in various disease states

Disease or Condition	Acute phase proteins assayed	Reference
Peripartum period	Hp, CRP, Fb Hp	[107] [108]
Lactation	Hp, CRP, Fb Hp, SAA Hp, M-SAA3, CRP Ceruloplasmin	[107] [108,139,529] [110,530] [112]
Neonatal period	Hp, SAA, LBP, AGP Hp, SAA, Fb Hp, SAA	[104] [109,220] [106,111,223,531]
Pasteurella multocida challenge	Hp, SAA, AGP Hp	[226] [228]&
Mannheimia hemolytica challenge	Hp, AMPs, inter-α-trypsin inhibitor Hp Hp, SAA, Fb Hp, Hp-MMP9	[176] [188]$ [177] [178]
Bovine viral diarrhea virus challenge	Hp, SAA, Fb	[177,179,181,182]
Bovine respiratory syncytial virus	Hp, SAA Hp, SAA, LBP, AGP	[227] (challenge) [187] (natural disease)
BRD – natural disease	Hp, SAA Hp Hp, LBP, transferrin Hp, Fb, α2- and γ-globulins, albumin Hp, SAA, LBP, AGP, Fb	[183] [184,222,225,228] [185] [235] [186]
Mastitis – experimental challenge models	Hp Hp, SAA Hp, SAA, LBP	[230] [229,231] [232]
Mastitis – natural disease	SAA, M-SAA3 Hp, milk Hp, SAA, M-SAA3 LBP	[191,532] (subclinical); [190,191] (clinical) [192] (subclinical) [193]
Metritis – natural disease	Hp	[116,196,197]
Endometritis – natural disease	Hp, SAA	[198-200]
Chronic inflammatory conditions	Hp, SAA Hp-MMP9 Hp, SAA, AGP	[533,534] [133] [527]
Metabolic disease	Hp, SAA Hp	[208] [113,197,210,211]

Disease or Condition	Acute phase protein assayed	Reference
Surgical disease	Hp, SAA Hp, SAA, Fb	208,209 207
Lameness – claw lesions	Hp Hp, SAA Hp, SAA, CRP, Fb	214 212 213
Vaccination	Fb, ceruloplasmin Hp	216,217 218
Transportation	Hp Hp, ceruloplasmin Hp, SAA	71* 535,536 537
Weaning	Hp, SAA, Lactoferrin Hp, Fb	538 189,539-541
Castration	Hp, ceruloplasmin	542

& BHV-1 and P. multocida challenge model

* preconditioning programs, weaning, and transport

weaning & transport

$ Mannheimia hemolytica model included effect of exposure to PI-BVDV

Product and Manufacturer Information

[a]http://www.destronfearing.com/equine.php

[b]https://www.embeddeddatasystems.com/

[c]http://www.fevertags.com/

[d]https://www.smaxtec.com/en/

[e]https://www.ecow.co.uk/

[f]http://americancalan.com/

[g]http://www.growsafe.com/

[h]https://www.lely.com/the-barn/feeding/cosmix/

[i]http://www.sacmilking.com/

[j]http://en.nedap-livestockmanagement.com/

[k]http://www.msr.ch/en/product/special_data_logger/rumination_sensor/

[l]http://www.scrdairy.com/

[m]https://www.lely.com/us/the-barn/milking/astronaut-a4/

[n]http://www.icerobotics.com/

[o]https://smartbow.com/en/solutions/wiederkaeu-ueberwachung.html

[p]https://www.cowmanager.com/en-us/

[q]http://www.milkline.it/

[r]https://www.cowmanager.com/en-us/

[s]https://www.delaval.com/en-us/

[t]https://www.agriculture-xprt.com/products/afiact-model-ii-cow-heat-detection-leg-tag-384463

[u]http://www.isense.farm/

[v]https://equinosis.com/

Chapter 2: Manuscript: Use of accelerometers to evaluate behavior changes in cattle administered continuous, low-dose endotoxin

J. E. Bayne,[*] P. H. Walz,[†1] T. Passler,[*] B. J. White,[‡] M. E. Theurer, [‡] and E. van Santen[#]

* Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, 36849

† Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, 36849

‡ Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, 66506

Department of Agronomy and Soils, College of Agriculture, Auburn University, Auburn, AL, 36849

The authors declare that there were no conflicts of interest. Funding was provided by the Auburn University Interdisciplinary Research and Equipment Grants Program. This manuscript represents a portion of a dissertation submitted by Dr. Bayne to the Auburn University Graduate School as partial fulfillment of the requirements for a Doctor of Philosophy degree.

[1] Corresponding author: walzpau@auburn.edu

ABSTRACT

Identification of illness and changes in behavior associated with morbidity through the use of remote data acquisition technologies, such as accelerometers, is an important area of on-going research for the cattle industry. Behavioral changes during continuous, low-dose lipopolysaccharide (LPS) administration in beef calves were objectively quantified using accelerometers, along with traditional markers of inflammation in a subclinical endotoxemia model. Osmotic mini-pumps (OMP) were placed subcutaneously in 20 conventionally-weaned beef calves in order to deliver 30 µg/(kg·d) of *E. coli* O55:B5 endotoxin (n = 10) or saline (n = 10) for 7 d. Rectal temperature, fibrinogen, albumin, and platelets were higher and BW lower in endotoxin-treated calves compared to control animals during the study period consistent with the presence of subclinical endotoxemia. Baseline and changes in behavioral indices during and subsequent to LPS-infusion were captured continuously throughout the 28-d study via an accelerometer affixed to the right hind limb of each calf. Mean baseline values for behavior indices were determined for 7-d prior to treatment with changes in behavior determined by expressing control and treatment values for the subsequent 21-d as a ratio of mean baseline values. Changes in behavior were observed within both treatment and control groups, relative to their respective baselines, with LPS-infused calves spending less time lying (d 11), increased time standing (d 11), and increased time spent walking (d 7, 11, and 14); whereas, saline-infused calves had decreased time lying (d 7, 9 to 11, 14 to18, and 20), increased time standing (d 0, 5, 7 to 20), and increased time spent walking (d 0, 7, and 14). Comparison of behavioral indices between groups demonstrated significant differences in all 3 behavioral indices. LPS-infused calves spent significantly more time lying (d 9, 12, 14, and 18), less time standing (d 9, 10, 12 to

20), and more time walking (d 15). The application of subcutaneously placed OMP to deliver exogenous LPS were well tolerated in calves, with successful elicitation of an acute phase response. Accelerometers demonstrate potential usefulness and sensitivity for the remote monitoring and detection of sickness behaviors in calves experiencing mild endotoxemia.

INTRODUCTION

During endotoxemia caused by gram-negative bacterial lipopolysaccharide (LPS), the host responds in an orchestrated series of events comprising the acute phase response (APR).[16,543] Characterization of physiological parameters and changes in inflammatory cells, pro-inflammatory cytokine expression, and acute phase proteins (APP), including haptoglobin, following IV LPS in cattle has been extensively reviewed.[544,545] The stereotypical sickness behaviors clinically recognizable during an APR include fever, lethargy, reduced feed intake, and weight loss.[18,24,50,546] Detection of illness in cattle relies heavily on subjective assessment, which is reported to have low diagnostic sensitivity and specificity.[237] Analysis of cattle behavior independent of human observation for the identification of morbid cattle earlier in disease onset is a growing area of interest in the cattle industry. Remote monitoring systems have demonstrated the capability of identifying morbid cattle under experimental and naturally occurring disease conditions, often predictive of illness before clinical signs are observed.[83,300,301,335,336,361,410] The objective of the present study was to assess the capabilities of accelerometers to objectively quantify changes in behavior during an APR in cattle administered continuous, low-dose LPS in a subclinical disease model. Behavior responses were evaluated in conjunction with traditional markers of inflammation, allowing further refinement of the impact of continuous, low-dose LPS administration on cattle behavior, and the validity of remote monitoring devices for the detection of subtle sickness behaviors in subclinical endotoxemia.

MATERIALS AND METHODS

Experimental Design

All procedures were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2011-2027). Twenty conventionally weaned, BVDV test-negative, mixed-breed beef calves that weighed between 162 and 200 kg were utilized in this study. Calves were weaned and castrated 60 d prior to study initiation and were maintained as 1 group on a biosecure pasture at the North Auburn Beef Unit (NABU). Calves were randomly assigned to CON (control, saline-infused, *n*=10) or LPS (treatment, LPS-infused *n*=10) by means of a random number generator (Microsoft Excel 2010, Redmond, WA). Calves were acclimated to the study pasture for 14 d prior to study initiation and both groups of calves were housed together on a 1-ha biosecure pasture throughout the 28-d trial at NABU. Water and Bermuda grass hay were provided ad libitum, and a 12% protein concentrate was fed once daily at a fixed time (0500 h) to allow for clinical score assessment. Otherwise, human observation and activity in the vicinity of the pasture were restricted to minimize the effect of human activity on the behavioral data collected.

The health of each calf was evaluated by 1 veterinarian (JEB) daily when the protein concentrate was fed. This veterinarian devoted a minimum of 30 min for evaluation of the health of the group and visually observed and assigned each calf a CIS on a scale of 1 to 4 (CIS; 1 = clinically normal, 2 = slightly ill, 3 = moderately ill, and 4 = severely ill) as previously described [462]. Implant sites were visually assessed for degree of swelling, dehiscence or presence of exudate. For any calf assigned a CIS of 3 or 4, a physical examination was performed and treatment was administered in accordance with the standard health protocols for the research facility. On d -7, calves were restrained in a chute, examined, and an accelerometer and

pedometer were attached to the right hind leg of each animal to continuously monitor activity throughout the 28-d trial. Subsequently, calves were restrained in a chute and examined at 7-d intervals (d -7, 0, 7, 14, and 21) in order to minimize periods of altered behavior as a result of restraint. Baseline behavior data were collected beginning on d -7.

On d 0, all calves were implanted with two osmotic mini-pumps (OMP; ALZET 2ML1 Osmotic Pump; Durect Corporation, Cupertino, CA) in order to continuously deliver low-dose LPS in adaptation of a model of systemic inflammation in sheep [547] and camelids [548]. Briefly, the calf was restrained in a chute and a 10 cm x 10 cm area was clipped and aseptically prepared over the left 8th to10th ribs, approximately 10 cm below the level of the transverse processes. Local analgesia was provide using 10 mL of 2% lidocaine hydrochloride. A 3 cm vertical incision was made and the subcutaneous tissue bluntly undermined to create a pocket of sufficient size for subcutaneous placement of 2 OMP. The skin incision was closed with skin staples. Each calf was implanted with 2 OMP, which were filled according to manufacturer instructions to deliver 10 µL/h volume for 7-d duration. Endotoxin (*E. coli* O55:B5, Sigma-Aldrich, St. Louis, MO) was delivered at a dose of 30 µg/(kg·24 h) (LPS, *n*=10) or physiologic saline alone (CON, *n*=10). Body weight determined on d -7 was used to calculate amount of endotoxin to be mixed and loaded into individualized OMP for each calf in order to deliver 30 µg/(kg·24 h). On d 7, the pumps were removed, using a similar protocol of site preparation, local anesthesia, and skin closure as described above.

On ds -7, 0, 7, 14, and 21, physical examinations were performed and blood was collected. Jugular venipuncture was performed for complete blood cell counts and serum biochemistry, with the collection of 20 mL whole blood in evacuated tubes containing the anticoagulant sodium EDTA and into tubes without anticoagulant, which were centrifuged (1200

x g, 30 min, room temperature) and serum removed. Haptoglobin concentrations were determined in samples collected on d 0 and 7.

Accelerometer-based behavior assessment

Commercially manufactured accelerometers (GP1 Programmable Accelerometer, Sensr Company, Elkander, IA), consisting of a tri-axial capacitance type +/- 10g integrated-circuit were attached to the lateral aspect of the right rear leg just proximal to the metacarpophalangeal joint in accordance with methods used in previous studies. [414,463] The accelerometer system consists of the GP1 SENSR unit contained inside a waterproof case which is padded and strapped to the leg using Velcro straps. The entire system weighs approximately 0.5 kg. The accelerometers contain two AA lithium batteries and 1 Mb data storage, so data can be collected continuously for a period of 7 d. Weekly downloading of data was based on a user-defined reporting interval (epoch) of 5 s and a memory storage capacity of 1 megabyte. Commercial data mining software (Insightful Miner, Insightful Corporation, Seattle, WA) was used to transform data into a uniform structure for comparison and analysis. As previously validated[414], individual animal behavior at each data point (epoch) is categorized as walking, standing, or lying. Data recorded by the accelerometers were downloaded on d 0, 7, 14, and 21 following removal of the mounting apparatus and accelerometer from the limb of restrained calves. The accelerometers were removed from the apparatus and connected to a laptop via a universal serial bus cable, and the data were downloaded from the accelerometers to the computer. Then, the accelerometer was replaced within the mounting apparatus and reaffixed to the limb of the calf. Behavioral data collected for 0.5 h before and 1 h after feeding and handling procedures were removed from analysis, as this potentially introduced bias into the data because handling directly influences behavior.

Clinical Pathology

Complete blood cell counts and serum biochemistries were performed using routine laboratory methods by the Clinical Pathology service of the College of Veterinary Medicine at Auburn University. Serum aliquots were frozen at -80° C following each blood sample collection for haptoglobin analysis. Haptoglobin concentrations were determined in sera using a commercially available colorimetric assay as instructed by the manufacturer (Phase Haptoglobin Assay, Tridelta Development Ltd., Maynooth, CO Kildare, Ireland). The analytical sensitivity of this multi-specific test, as given by the manufacturer, is 0.005 mg/mL of haptoglobin. Briefly, 7.5 µl of sample or calibrator standards were added in duplicate to wells of 96-well microtiter plates. Five minutes subsequent to addition of both reagents, the optical density of each well was determined by a microplate reader at 615 nm wavelength.

Data Analysis

Sample size computation and power analysis was performed by software program (G*Power 3.1.2, Heinrich Heine University Dusseldorf, Dusseldorf, Germany). Power and sample size calculations were performed by using the *a priori* function for proportional testing [549]. The following assumptions were made: the probability of a type I error was chosen as $\alpha = 0.05$, desired power was set to at least 0.90, number of groups = 2. The assumed proportion of walking for the control group was 0.2 and for the LPS group was 0.1. The calculated total sample size based on described assumptions was 16 calves ($n = 8$ per group), and calculated power was 0.95. To account for potentially missing data points due to data loss with improperly positioned accelerometers or the need to discard data during unforeseen handling events, 10 calves per group were included in this study.

Clinical laboratory data were analyzed using mixed models procedures as implemented in commercial software (SAS® PROC GLIMMIX, version 9.2, SAS Institute Inc., Cary, N.C.), where treatment and time after treatment and interaction between treatment and time were considered to be fixed effects. Because the experimental design had a repeated nature, we modeled the residual covariance structure comparing likely models such as unstructured and first-order autoregressive; the best-fitting model was chosen on the basis of the corrected Akaike's Information Criterion. Treatment groups were compared at each time point because there was a significant interaction.

For accelerometer data, the average cumulative daily activity for each group during d -7 to -1 was used as the baseline value in the analysis; control or LPS-infusion started on d 0. Cumulative daily activity for each trial date was converted to a ratio with the baseline value as denominator. Generalized mixed models procedures with a lognormal distribution function as implemented in commercial software was used to analyze the data; treatment and time after treatment and time interaction were considered to be fixed effect. Because the experimental design had a repeated nature, we modeled the residual covariance structure comparing likely models such as unstructured and first-order autoregressive; the best fitting model was chosen on the basis of the corrected Akaike's Information Criterion. Least squares means and confidence intervals were back-transformed to the original scale. Because the natural log of one equals zero, the standard output in commercial software, which tests whether a given mean is significantly different from zero, effectively tests whether the activity on a given day differs significantly from the baseline. Pairwise contrasts between CON and LPS were calculated for each trial day.

Baseline data from calves demonstrating extreme values for each behavioral index (percentage of time spent walking, standing, or lying) were discarded from data analysis. To accomplish this,

individual calf behavior over the 7-d baseline period were evaluated in relation to the baseline behavior data from cohorts. Data were removed prior to analyses if an individual calf's baseline behavior data for any of the 3 indices (walking, standing, or lying) were greater than 3 SD away from the mean of the cohort.

RESULTS

Clinical Illness Scores (CIS)

All calves were clinically normal (CIS 1) during the 7 d prior to OMP placement. Mild clinical illness (CIS 2) was observed in one LPS calf on d 1 after OMP placement. All calves scored a CIS of 1 thereafter for the remainder of the 28-d study. No animal required treatment for clinical illness during the 28-d period. A right forelimb lameness (2/5) was observed in 1 CON calf starting on d 1, which progressively improved and had resolved by d 3; data from this calf were included in all analyses as it did not deviate from the baseline behavior of the group. No skin lesions or lameness associated with accelerometer placement were observed in any of the calves throughout the study.

Physical examination and clinicopathological findings

Osmotic mini-pump placement was well tolerated by all calves, with no significant swelling, dehiscence or discharge noted from surgical implantation sites from d 0 – 7. Although no dehiscence or purulent exudate were observed at OMP implant sites, mild swelling of the OMP site was observed in several calves. Starting on d 8, one d after OMP removal, 2 LPS calves displayed mild swelling, with resolution in one calf the following day. The other calf demonstrated mild swelling of the site until d 18 of the trial. An additional LPS calf developed very mild swelling on d 11, which persisted until d 13. Three CON calves developed very mild

swelling which lasted in duration for one d (d 12 and 13, respectively) and two day (d 17 and 18). No dehiscence or suppurative exudate were observed in any of the calves for the duration of the study.

Rectal temperatures varied significantly between groups ($P = 0.021$) and over time ($P < 0.001$) during the study, but a significant treatment by day interaction was not detected ($P = 0.136$). In LPS calves, significantly elevated rectal temperatures were observed on d 21 compared to CON calves (40.2°C vs. 39.8°C, respectively; $P = 0.0061$). LPS calves tended to exhibit increased rectal temperatures on d 7 compared to CON calves (40.1ºC vs. 39.8ºC, respectively; $P = 0.0647$). On d 21, significantly greater BW were observed in CON calves compared to LPS calves (225 kg vs. 215 kg, respectively; $P = 0.0486$). On day 7, a trend for greater BW in CON calves compared to LPS calves (206 kg vs. 197 kg, respectively; $P = 0.0728$).

No significant treatment effects were found in total white blood cell counts, neutrophil count, lymphocyte counts, or serum iron concentrations between LPS and CON calves throughout the 28-d trial. Lymphocyte counts and serum iron concentrations varied significantly over time ($P = <0.001$ and 0.0004, respectively). Platelets were significantly higher in LPS calves on d 7 compared to CON calves ($P = 0.018$). A significant treatment by day interaction was observed for serum fibrinogen concentrations ($P = 0.0039$), with a greater increase in fibrinogen concentration from d 0 to d 7 in LPS calves, followed by a gradual decrease in both CON and LPS groups by d 21. Serum fibrinogen was significantly greater in LPS calves on d 7 compared to CON calves (750 mg/dl vs. 550 mg/dl, respectively; $P < 0.0001$). Fibrinogen tended to be greater in LPS calves compared to CON calves on d 0 and d 14 ($P = 0.072$ and 0.070, respectively). Significantly lower albumin concentrations were found in LPS calves compared to

CON calves on d 0, 7, 14, and 21 (P = 0.007, < 0.01, 0.008, and 0.007, respectively). Haptoglobin concentrations varied significantly over time (P = 0.009), but not between groups.

Behavior data

Behavioral data from one LPS calf was removed due to baseline walking behavior being greater than 3 SD from the LPS mean for baseline walking behavior. Data for the other behaviors from this calf was included in the other analyses. All behavioral data from all calves were included in data analysis following OMP implantation and removal, as all met inclusion criteria. Following the placement and subsequent removal of the OMPs, significant changes from baseline behavior in LPS calves included decreased time spent lying on d 11 (P = 0.028; Fig. 1A), increased time spent standing on d 11 (P = 0.013; Fig. 1B), and increased time spent walking on d 7, 11, and 14 (P = 0.003, 0.045, and 0.016, respectively; Fig. 1C). Following the placement and subsequent removal of the OMPs, significant changes from baseline behavior in CON calves included decreased time spent lying on d 7, 9 to 11, 14 to 18, and d 20 ($P \leq 0.002$; Fig. 1A), increased time spent standing on d 0, 5, 7 to 20 ($P \leq 0.03$; Fig. 1B), and increased time spent walking on d 0, 7, and 14 (P = 0.035, 0.003, and 0.014, respectively; Fig. 1C). A decrease in time spent walking compared to baseline behavior was observed in CON on d 15 (P = 0.041; Fig. 1C). Comparing changes from baseline behavior between groups, significant differences between CON and LPS calves were observed in all 3 behavioral indices. The time spent lying was significantly different on d 9, 12, 14, and 18, with CON calves spending significantly less time lying compared to LPS calves (P = 0.033, 0.036, 0.043, and 0.041, respectively; Fig. 1A). The time spent standing was significantly different on d 9, 10, 12 to 20, with CON calves spending significantly more time standing compared to LPS calves ($P \leq 0.044$; Fig. 1B). The time spent walking was significantly different on d 15, with CON calves spending significantly

less time walking whereas LPS calves demonstrated a significant increase in the time spent walking ($P = 0.038$; Fig. 1C).

DISCUSSION

The major objective of the present study was to assess the capabilities of remote behavioral data collection using accelerometers in cattle in a subclinical endotoxemia model. In contrast to previous IV bolus challenge models which induce moderate to severe clinical signs and pathological events[545], the present study utilized osmotic mini pumps (OMP) to deliver a continuous, low-dose LPS infusion over several days. Use of OMP to deliver LPS resulted in detectable sickness behavior in LPS calves compared to CON calves. Calves in both groups demonstrated a change from their respective baseline behavior following OMP removal. LPS calves spent less time lying and increased time spent standing on d 11, and increased time walking on d 7, 11, and 14 compared to baseline. CON calves spent less time lying, more time standing, and increased time spent walking for the majority of days following OMP removal. A pen effect of treatment group is not expected to be present in this study as all calves were housed together on the same pasture. The trends observed in time spent lying, standing, and walking during the course of the study occurred concomitantly in both groups of calves, however, significant differences were observed between groups and likely represent the effects of LPS-infusion.

Comparison of behaviors demonstrated that LPS calves spent significantly more time lying and significantly less time standing compared to CON calves. The sickness behaviors observed in LPS calves in the present study are in agreement with previous experimental endotoxemia challenge studies. In a severe LPS challenge model (0.5 µg/kg BW, IV) in 4-wk old calves, marked behavioral changes included marked depression, complete anorexia, respiratory

distress, and recumbency.[545] Worsening of clinical signs, predominantly the degree of depression, coincided with the onset of the febrile response, and resolution of sickness behaviors was observed at a minimum 6 h post-LPS administration.[545] In a low-dose (0.025 to 0.05 µg/kg BW) LPS challenge study, 3-week old calves demonstrated measureable behavioral changes that preceded the onset of fever.[550] Behavioral changes included decreased rumination, hay consumption, and grooming behavior. Although no changes in the overall time spent standing or lying were observed, a significant increase in inactivity exemplified by increased lying and standing bouts was found.[550] Werling et al. (1996) and Steiger et al. (1999) utilized a prolonged, low-dose infusion of LPS (2 µg/kg BW, IV over 100 min) to produce an APR in heifers, more closely simulating naturally occurring gram-negative bacterial disease. Physiological and behavioral changes included reduced feed intake, increased body temperature, and marked metabolic changes in heifers, with maximal feed intake suppression observed at 4 h and which continued for 24 h post-infusion.[551,552] Even lower doses of LPS administration, for example 10 ng/kg in adult dairy cows, elicit acute onset of depression, anorexia, rumen hypomotility and diarrhea, highlighting the sensitivity of cattle to endotoxin.[553] However, it is unlikely that the time course of changes in LPS concentrations in blood after IV bolus injection and the acute onset, short duration of clinical signs truly mirror the time course of naturally occurring disease. Continuous, low-dose LPS delivery to cattle using OMP is a viable option as shown in this study.

The use of OMP in order to deliver a continuous, low-dose infusion of LPS to cattle as well as the characterization of behavior changes using remote, objective behavior monitoring technology in this LPS model is unique in its approach. The devices offer utility in the route of endotoxin delivery and uptake by the body, as they can be attached to indwelling intravenous and intraperitoneal catheters or directly implanted in the peritoneal cavity or subcutaneous tissue.[554-

[559] Use of OMP allows characterization of the acute phase response to chronic LPS exposure without the severe clinical signs and APR associated with IV bolus administration of LPS, and more closely mirrors the conditions experienced under naturally occurring gram negative bacterial infection. Use of OMP has predominately been used in laboratory rodents, with limited application to larger livestock species.[547,548] Recently, OMPs were utilized to deliver a continuous, low-dose LPS infusion to refine the current knowledge of inflammatory markers best suited to characterize the acute phase response in alpacas.[548] The present study is unique in utilizing OMP in cattle, extending the time frame of LPS delivery even further than previous studies.

One aim of the present study was to elicit an acute phase response by exogenous LPS administration while causing only subclinical to very mild clinical signs, in order define the ability of accelerometers to objectively quantify behavior changes under such conditions. Similar to the subclinical nature produced in alpacas[548], the clinical scores of LPS-infusion calves in the present study remained normal throughout the study period. Observed changes in clinicopathological variables support the induction of an acute phase response in LPS calves. These included a significant increase in fibrinogen, which is considered a positive acute phase protein, on day 7, and a significant decrease in albumin, a negative acute phase protein, on d 7, 14, and 21. In addition, LPS calves had significantly higher platelet counts compared to CON calves on d 7. However, the endotoxin challenge potentially was insufficient in its magnitude, as significant differences in white blood cell counts, serum iron, and haptoglobin were not observed between LPS and CON calves as expected.

To evaluate the ability of accelerometers to detect sickness behavior, human observations, which alter cattle behavior, were limited to once daily clinical assessment and once

every 7 d for data retrieval and sample collection. Thus, lack of repeated sampling is a limitation of the present study and may account for the lack of detection in changes of peripheral white blood cells and serum biomarkers. White blood cell and acute phase protein changes during an acute phase response are transient and could have returned to baseline levels between d 0 and 7, going undetected in LPS calves, based on the limited weekly sampling interval. Alternatively, the dose of endotoxin delivered was conceivably too small to fully bring about a physiologically relevant APR. Although not feasible in the present study, further confirmation of successful LPS delivery to calves using OMPs could include the measurement of circulating endotoxin levels, as well as the quantification of residual LPS in OMPs upon their removal as these devices have not been previously validated for subcutaneous delivery of LPS in cattle. Determination of these factors in the future would further substantiate the use of OMPs to deliver continuous, low-dose LPS infusion in cattle. Surgical placement of OMPs in the subcutis were well tolerated in all calves throughout the infusion period and no long-term effects such as abscess formation or implantation site dehiscence were observed following their removal for the duration of the study period. Additional evidence that an endotoxemia was successfully achieved by OMP LPS-delivery was the significant difference in BW observed at day 21, where LPS calves were significantly lighter compared to CON calves. Changes in BW during or following short-term, intravenous bolus LPS administration has not always been a feature in previous studies. This most likely reflects a short duration of study observation, thereby minimizing the potential to detect significant weight loss as well as the limited impacts of LPS administration on overall feed intake in calves in these studies.[550,552] The present study was longer in duration, allowing quantification of LPS effects post-infusion, and potentially more similar to endotoxemia during gram negative bacterial infection under natural conditions. The present findings are in agreement

with those of Theurer et al., which found that calves challenged with *Mannheimia haemolytica* had significantly greater BW loss compared to control calves, spending less time at feed bunk, hay source, and water after inoculation.[412] In part, weight differences in endotoxemic calves may reflect alterations in feed intake characteristic of sickness behavior and the resultant catabolic state during LPS-infusion, including a decrease in tissue protein accretion and increased amino acid catabolism to support APP synthesis, immune cells, and glucogenic precursors during inflammation.[560]

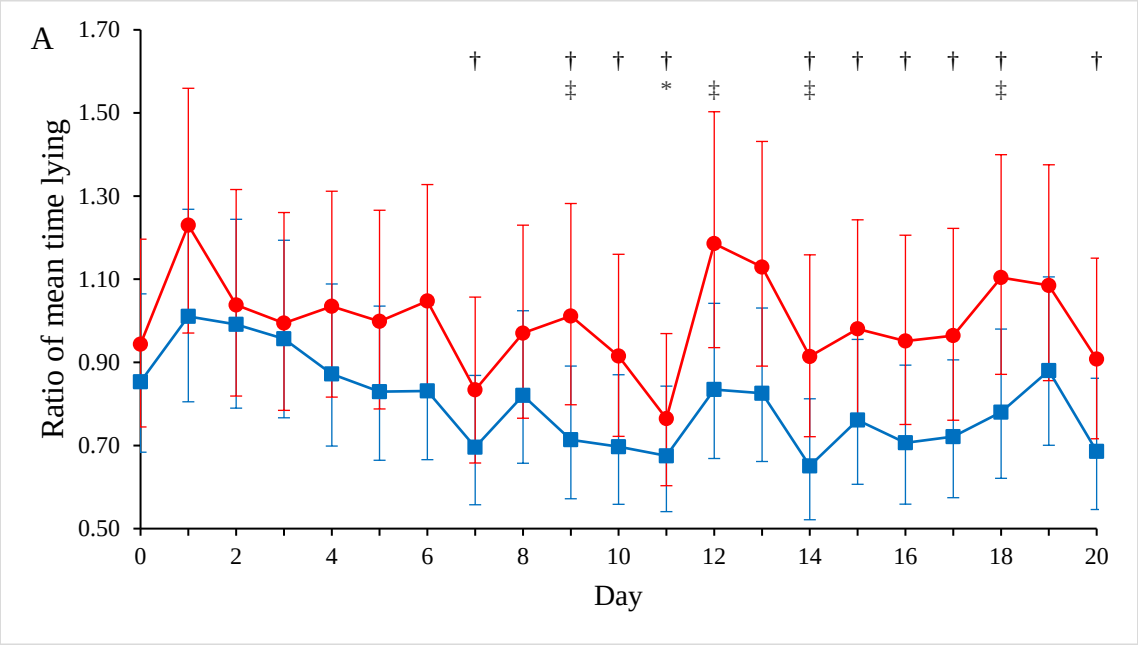
The ability of accelerometers to detect subtle sickness behaviors in LPS-infused calves herein are also in agreement with previous studies which have utilized several different remote monitoring technologies to quantify sickness behavior. Following endoscopic pulmonary inoculation with *Mannheimia haemolytica* in which clinically detectable respiratory disease was evident, calves spent significantly more time lying and less time standing as determined by accelerometers, and took fewer steps following bacterial inoculation compared to baseline as determined by pedometers.[462] Significant reductions in activity level, including proximity to feed bunks, and less distance traveled have been observed following intranasal inoculation of *Mycoplasma bovis* with the application of a remote location monitoring device.[411] In a recent study by Bayne et al., subtle sickness behaviors following bovine viral diarrhea virus (BVDV) challenge were demonstrable through the use of accelerometers in a mild BVDV infection model, whereby BVDV-infected calves spent less time standing during the period of viremia.[463] However, the ability to distinguish infected and non-infected calves through the use of accelerometers was limited.[463] Accelerometers have also recently demonstrated the ability to detect sick animals experiencing naturally acquired disease in a commercial field setting.[464]

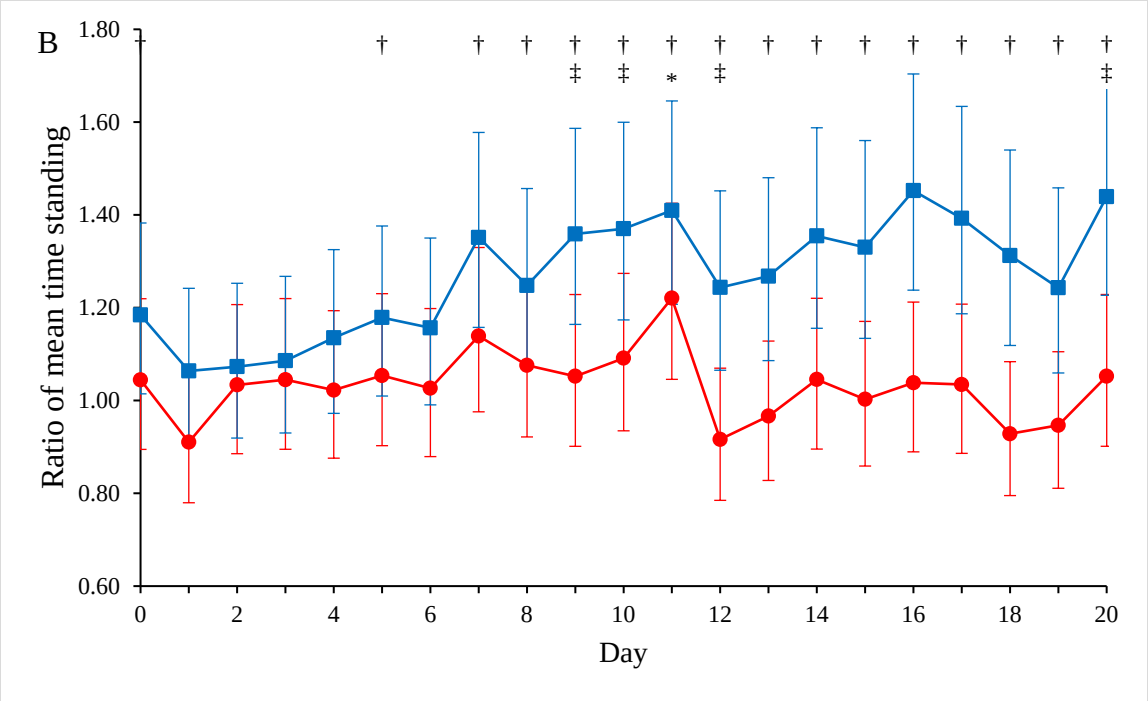
These studies, along with the present results, demonstrate the ability of these remote activity monitoring technologies to detect sickness behaviors in cattle.

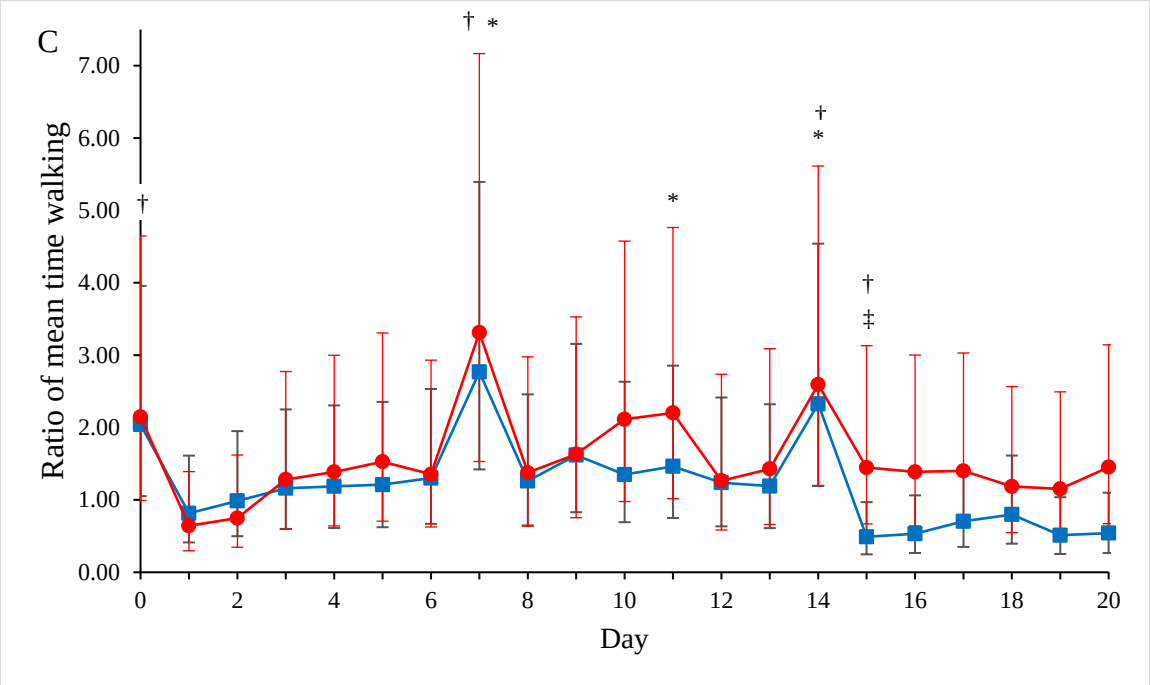
The present report demonstrates the potential usefulness and sensitivity of accelerometers for remote monitoring of sickness behavior in calves experiencing mild endotoxemia. The application of subcutaneously placed OMP were well tolerated in calves, successfully elicited an acute phase response while remaining subclinical in nature in LPS-infused calves.

Figure 1.

Ratio of the mean time spent lying (A), standing (B), or walking (C) daily to baseline (mean time spent lying, standing, or walking during the 7 d before experimental infusion) for CON calves (control; saline-infused; *n* = 10; blue line, squares) and LPS calves (treatment; LPS-infused; *n* = 10; red line, circles). LPS calves were administered *E. coli* O55:B5 endotoxin at 30 µg/(kg·24h) for 7 d by use of a subcutaneously placed osmotic mini-pump (OMP) on study d 0. A comparable volume of physiological saline was administered to control calves in a similar fashion. Removal of OMP occurred on d 7. The brackets delimit the range for each ratio on a given day. The values for CON and LPS calves represent the means for 10 calves. †Within a day, the mean time spent on the given behavior differs significantly ($P < 0.05$) from baseline for the CON group. *Within a day, the mean time spent on the given behavior differs significantly ($P < 0.05$) from baseline for the LPS group. ‡Within a day, the ratio for the LPS group differs significantly ($P < 0.05$) from the CON group.




B
Ratio of mean time standing
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0
2
4
6
8
10
12
14
16
18
20
Day


C
Ratio of mean time walking
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
2
4
6
8
10
12
14
16
18
20
Day

Chapter 3: Evaluation of behavioral changes in cattle using three-dimensional accelerometers during experimental infection with bovine viral diarrhea virus

Chapter formatted as published in *AJVR*, 2016, 77(6), 589-596

Jenna E. Bayne, DVM, DACVIM[1], Paul H. Walz, DVM, PhD, DACVIM[1*], Thomas Passler, DVM, PhD, DACVIM[1], Brad J. White, DVM, MS[2], Miles E. Theurer, DVM, PhD[2], and Edzard van Santen, PhD[3]

[1] College of Veterinary Medicine, Departments of Clinical Sciences and Pathobiology, Auburn University, Auburn, AL 36849, USA

[2] College of Veterinary Medicine, Department of Clinical Sciences, Kansas State University, Manhattan, KS 66506, USA

[3] Department of Crop, Soils and Environmental Sciences, College of Agriculture and Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849 USA

Supported by American Association of Bovine Practitioners and Auburn University Interdisciplinary Research and Equipment Grants Program.

This manuscript represents a portion of the dissertation submitted by Dr. Bayne to the Auburn University Graduate School as partial fulfillment of the requirements for Doctor of Philosophy degree.

Presented in part at the American Association of Bovine Practitioners Annual Conference, Milwaukee, WI, September 2013.

*Corresponding author: Dr. Walz at walzpau@auburn.edu

The authors declare that they have no competing interest.

The authors wish to express their appreciation to the American Association of Bovine Practitioners and Auburn University Interdisciplinary Research and Equipment Grants Program for funding support. The authors are also grateful for the valuable technical assistance from Ms. Yijing Zhang and Ms. Pat Galik. Assistance with animal care and handling provided by Mr. Steven Ledbetter, Mr. George Fincher, Mr. Jon Whitlock, and Dr. Kay Riddell is likewise greatly appreciated.

Abstract

Objective – To determine the ability of remote behavioral data collection via accelerometers in conjunction with conventional methods of clinical assessment and traditional markers of inflammation to detect changes in a low-virulent BVDV challenge model compared to non-challenged controls.

Animals – 20 beef steer calves (mean weight: 238 kg).

Procedures – All calves were fitted with an accelerometer for remote continuous behavioral data recording (standing, lying, and walking). Calves were randomly assigned to 2 treatment groups (BVDV or sham inoculation). Behavioral data, rectal temperature, weights, and blood samples were collected on days -7, 0, 7, 14, 21, and 28. Subjective assessment was limited to once daily throughout the 35-day trial.

Results – Total WBC and neutrophil counts were significantly lower in BVDV calves on day 7 and 14 compared to controls. A significant decrease in lymphocyte counts in BVDV calves was observed on day 7. No significant differences were observed between groups with respect to haptoglobin, fibrinogen, or serum iron. Following inoculation, BVDV calves spent decreased

time walking and increased time lying and standing, compared to their baseline behavior, with the exception of decreased standing on day 8. Similar changes in lying and walking time were observed in control calves following sham-inoculation. Control calves spent less time standing compared to baseline behavior following sham inoculation. However, when contrasts were made between groups, BVDV calves were found to spend significantly less time standing on day 8 post-inoculation compared to control calves.

Conclusions and Clinical Relevance – Results indicate that, following low-virulent BVDV inoculation, changes in behavior did not differ between BVDV and control calves, but subtle changes and overall trends were demonstrable via accelerometers during the 35-day trial period.

Abbreviation List

BRD: Bovine respiratory disease

BVDV: bovine viral diarrhea virus

CIS: Clinically illness score

EMEM: Eagle's minimum essential medium

PI: persistently infected

Mb: Megabyte

MDBK: Madin-Darby bovine kidney

SD: Standard deviation

TCID: Tissue culture infective dose

VI: Virus Isolation

VN: Virus neutralization

WBC: White blood cells

Introduction

Bovine respiratory disease continues to be one of the most important diseases of cattle and results in enormous financial losses. Bovine respiratory disease is a multifactorial disease and adverse management factors and different bacterial and viral pathogens contribute to the pathophysiology of disease. The role of BVDV in the causation of BRD is well documented, particularly as an immunosuppressive agent and potentiator of other BRD pathogens.[561,562]Immunosuppression results in both quantitative and functional alterations of the innate and adaptive immune systems.[563-566]The prevalence of PI cattle in feedlots on-arrival has been reported to be from 0.15% to 0.4%.[561,567]These PI cattle serve as a source for acute BVDV infection in cohorts. Acute infection with BVDV can cause a number of different disease syndromes, ranging from subclinical disease to peracute fatal diarrhea and reproductive failure.[568] In addition to immunosuppression, BVDV's role in BRD development can be due to pneumonitis and the development of clinical signs due to systemic inflammation and pneumonic damage.[569]

Currently, detection of BRD relies upon subjective assessment of cattle, which is reported to have low diagnostic sensitivity and specificity.[237] Cattle alter behaviors in the presence of human observers, and clinical signs of BRD may not be detected until significant pathology has developed. Efforts to identify BRD and predict its occurrence have utilized physical examination, auscultation of the lungs, and clinicopathologic measures of inflammation, including alterations in inflammatory cell counts and concentrations of acute phase proteins, such as haptoglobin.[462] These diagnostics are invasive, expensive, and are subject to variability dependent upon the stage of disease. A delay in disease recognition hinders successful treatment outcomes and the appropriate use of therapeutics. [462] Furthermore, the inability to reliably detect

mild disease could lead to an underestimation of the production-limiting impact of sickness behavior in cattle.[50]

Technological advancements in animal care allow remote collection of objective measures for a number of physiological parameters in cattle in real-time. Changes in body temperature, utilizing orbital thermography[334-336] and remote rumen boluses[301,302] were found to be predictive of disease onset in advance of subjective assessment parameters under field conditions. Analysis of cattle behavior independent of human observation as an aid in early disease detection has received increasing interest by the research community and cattle industries, but these technologies need further investigation. Research evaluating cattle behaviors, such as feeding and drinking have revealed a distinct relationship between those behaviors and unfavorable outcomes with respect to animal health.[17,83] Cattle activities, such as standing, lying, feeding, and drinking, have been evaluated in their use for the identification of sickness in cattle. Accelerometers are small and noninvasive devices that are used to objectively monitor animal behavior, with minimal impact on inherent behavior patterns and have been validated and used to describe behaviors in beef[409,414] and dairy cattle,[570] as well as dairy calves.[420] Along with pedometers, as a measure of overall activity levels, accelerometers were utilized in different BRD disease models for the characterization of sickness behavior in cattle.[411,412,462] In contrast to previous challenge models which induced moderately severe clinical signs and pathological effects, the main objective of the present study was to assess the capabilities of remote behavioral data collection using accelerometers in cattle inoculated with a low-virulent strain of BVDV in a subclinical disease model. Behavior responses were evaluated in conjunction with traditional markers of inflammation, allowing further refinement of the

impact of BVDV infection on cattle behavior, and the validity of remote monitoring devices for the detection of subtle sickness behaviors in subclinical BVDV disease.

Materials and Methods

Experimental Design—All procedures were approved by the Auburn University Institutional Animal Care and Use Committee (2011-2027). Twenty weaned, mixed-breed beef calves, weighing between 216 – 262 kg, were obtained from a single source and determined to be free of BVDV and exposure to BVDV by VI and VN testing, respectively. Calves had been weaned and castrated 60 days prior to the start of the study. Calves were sourced from a BVDV-free research herd and maintained on a single biosecure pasture at the North Auburn Beef Unit prior to acclimation to study pastures and study initiation. Calves were randomly assigned to BVDV (n = 10 calves) or control (n = 10 calves) groups. Calves were acclimated to their study pastures for 14 days prior to study initiation and were housed in 2.5 acre biosecure pastures at the North Auburn Beef Unit and North Auburn BVDV Unit, respectively, throughout the 35-day trial. Water and Bermuda grass hay were provided ad libitum, and a 12% protein concentrate was fed once daily at a fixed time (05:00) to allow for clinical score assessment. Otherwise, human observation and activity did not occur so as not to influence behavioral data collected. For biosecurity reasons, the veterinarian responsible for clinical score assessment and feeding was not blinded to treatment group.

At feeding time, the health of each steer was evaluated by a veterinarian, who devoted a minimum of 30 min/feeding to evaluate the health of each steer via visual inspection and assign a CIS (1 = clinically normal, 2 = slightly ill, 3 = moderately ill, and 4 = severely ill)[462]. If deemed necessary, a physical examination was performed on clinically ill steers and treatment was administered in accordance with the typical health protocols for the research facility (calves with

CIS of 3 or 4). On day -7, calves were restrained in a chute, examined, and an accelerometer was attached to the right hind leg of each animal to continuously monitor activity throughout the 35-day trial. Subsequently, calves were restrained in a chute and examined at 7-day intervals (days -7, 0, 7, 14, 21, and 28) in order to minimize periods of altered behavior as a result of restraint. Baseline behavior data were collected beginning on day -7. On day 0, control group calves were sham-inoculated, followed by the intranasal inoculation of the BVDV group calves with BVDV. All subsequent procedures were carried out with the handling of the control group before the BVDV group to prevent inadvertent exposure of controls to BVDV. On days -7, 0, 7, 14, 21, and day 28, physical examinations were performed and blood was collected. Jugular venipuncture was performed for complete blood cell counts and serum biochemistry. Haptoglobin concentrations were determined in samples collected on days 0 and 7. Virus isolation was performed on days 0, 7, 14, 21, and 28; VN on day 0 and 28. Data recorded by the accelerometers were downloaded on days 0, 7, 14, 21, and 28 following removal of the mounting apparatus and accelerometer from the limb of restrained calves. The accelerometers were removed from the apparatus and connected to a laptop via a universal serial bus cable, and the data were downloaded from the accelerometers to the computer. Then, the accelerometer was replaced within the mounting apparatus and reaffixed to the limb of the calf.

Inoculation of calves—On study day 0, all calves were administered by intranasal aerosol administration either 10^6 $TCID_{50}$ / mL of the BVDV 2 strain 134F for BVDV group calves or BVDV-free media for control group calves. The noncytopathic BVDV 2 strain 134F had been previously used by the investigators and was demonstrated to induce mild clinical disease. The BVDV strain was propagated in MDBK cells in EMEM,[a] supplemented with 10% equine serum,[b] L-glutamine,[a] penicillin G[a] (100 units/ml), and streptomycin[a] (100μg/ml). Virus was

harvested from cells by a single freeze-thaw method, aliquoted and stored (-80°C) until needed. The viral titer was enumerated prior to inoculation of calves.[571] For inoculation, an intranasal cannula was inserted in each nostril of the calf and a volume of 2 mL per nostril of instillation containing virus or BVDV-free media was given.

Accelerometer-based behavior assessment—Commercially manufactured accelerometers,[c] consisting of a tri-axial capacitance type +/- 10 *g* integrated-circuit were attached to the lateral aspect of the right rear leg just proximal the metacarpophalangeal joint in accordance with methods used in previous studies[409]. The accelerometer system consists of the accelerometer unit contained inside a waterproof case which is padded and strapped to the leg using Velcro straps. The entire system weighs approximately 0.5 kg. The accelerometers contain two AA lithium batteries and 1 Mb data storage, so data can be collected continuously for a period of 7 days. Weekly downloading of data was based on a user-defined reporting interval (epoch) of 5 seconds and a memory storage capacity of 1 Mb. Commercial data mining software[d] was used to transform data into a uniform structure for comparison and analysis. As previously validated,[409] individual animal behavior at each data point (epoch) is categorized as walking, standing or lying. Accelerometer data were continuously collected and downloaded at 7 day intervals, which entailed handling and restraint of the cattle on days 0, 7, 14, 21, and 28. Behavioral data collected for 0.5 hours before and 1 hour after feeding/handling procedures were removed from analysis, as this introduces bias into the data because handling directly influences behavior. Clinical Pathology—CBC and serum biochemistries were performed using routine laboratory methods by the Clinical Pathology service of the College of Veterinary Medicine at Auburn University. Serum aliquots were frozen at -80° C following each blood sample collection for haptoglobin analysis following conclusion of the experiments. Haptoglobin concentrations

were determined in sera using a commercially available colorimetric assay as instructed by the manufacturer.[e] The analytical sensitivity of this multispecific test, as given by the manufacturer, is 0.005 mg/ml of haptoglobin. Briefly, 7.5 µl of sample or calibrator standards were added in duplicate to wells of 96-well microtiter plates. Five minutes subsequent to addition of both reagents, the optical density of each well was determined by a microplate reader at 615 nm wavelength.

Virus neutralization—The standard VN microtiter assay was used to detect antibodies in the serum of calves. After heat-inactivation at 56°C for 30 minutes, serial, 2-fold dilutions (1:4 up to 1:2048) were made in 50 µL of culture medium. For each dilution, 3 wells of a 96-well plate were inoculated with an equal volume (50 µL) of culture medium containing 100 $TCID_{50}$ of the NCP BVDV 2 strain 134F, which is the BVDV strain used for the inoculation of calves in the present study. After inoculation, the plate was incubated (38.5°C) in a humidified atmosphere of 5% CO_2 and air for 1 hour. Then, 2.5 x 10^3 MDBK cells in 50 µL of culture medium were added to each well. Plates were incubated for 72 hours and immunoperoxidase labeling of the cell monolayers was performed to detect neutralization of the virus. Anti-BVDV antibody titer herein is expressed as the greatest dilution of sample at which at least 2 of 3 wells are free of immunoperoxidase staining.

Virus Isolation—Whole blood collected in evacuated tubes containing sodium EDTA was processed by hypotonic lysis of the red blood cells to yield the WBC fraction. The isolated WBCs were resuspended in EMEM containing 10% equine serum, L-glutamine, and antibiotics. The cell suspension underwent co-cultivation in 25 cm^3 flasks containing monolayers of MDBK cells and was incubated for 5 days at 37°C and 5% CO_2. Following cultivation, 50 µl of the cell culture supernatant was inoculated in triplicate into wells on 96-well microtiter plates containing

monolayers of MDBK cells in EMEM containing 10% equine serum and antibiotics. After 3 days incubation at 37°C and 5% CO_2, the cells were stained for viral infection using an immunoperoxidase staining assay to detect noncytopathic biotypes of BVDV.

Data Analysis—Sample size computation and power analysis was performed by software program.[f] Power and sample size calculations were performed by using the *a priori* function for proportional testing [549]. The following assumptions were made: the probability of a type I error was chosen as $\alpha = 0.05$, desired power was set to at least 0.90, number of groups =2. The assumed proportion of walking for the control group was 0.2 and for the BVDV group was 0.1. The calculated total sample size based on described assumptions was 16 calves (n = 8 per group), and calculated power was 0.95. To account for potentially missing data points due to data loss with improperly positioned accelerometers or the need to discard data during unforeseen handling events, 10 calves per group were included in this study.

Clinical laboratory data were analyzed using mixed models procedures as implemented in commercial software,[f] where treatment and time and interaction between treatment and time were considered to be fixed effect. Because the experimental design had a repeated nature we modeled the residual covariance structure comparing likely models such as unstructured and first-order autoregressive; the best-fitting model was chosen on the basis of the corrected Akaike's Information Criterion. Treatment groups were compared at each time point because there was a significant interaction.

For accelerometer data, the average cumulative daily activity for each animal during days -7 to -1 was used as the baseline value in the analysis; sham or BVDV inoculation occurred on day 0. Cumulative daily activity for each trial date was converted to a ratio with the baseline value as numerator. Generalized mixed models procedures with a lognormal distribution function

as implemented in commercial software[g] was used to analyze the data; treatment and time after treatment and time interaction were considered to be fixed effect. Because the experimental design had a repeated nature we modeled the residual covariance structure comparing likely models such as unstructured and first-order autoregressive; the best-fitting model was chosen on the basis of the corrected Akaike's Information Criterion. Least squares means and confidence intervals were back-transformed to the original scale. Because the natural log of one equals zero, the standard output in commercial software,[g] which tests whether a given mean is significantly different from zero, effectively tests whether the activity on a given day differs significantly from the baseline. Pairwise contrasts between the control and BVDV groups were calculated for each trial day.

Baseline data from calves demonstrating extreme values for each behavioral index (percentage of time spent walking, standing, or lying) were discarded from data analysis. To accomplish this, individual calf behavior over the 7-day baseline period were evaluated in relation to the baseline behavior data from cohorts. Data were removed prior to analyses if an individual calf's baseline behavior data for any of the 3 indices were greater than 3 SD away from the mean of the cohort.

Results

Clinical Illness Scores (CIS) — All BVDV calves were clinically normal (CIS 1) during the 7 days prior to inoculation. In 2 control calves, CIS of 2 were recorded on days -3 and -2. Mild clinical illness (CIS 2) was observed in 4/10 BVDV calves on days 8 through 12 post-inoculation, and in 1,3, and 2 BVDV calves on days 14, 17, and 23, respectively. In the control group, CIS of 2 were observed on days 15 and 16 post-inoculation in 2 calves and in 1 calf, respectively. No animal required treatment for clinical illness during the 35-day study period, as

no animal demonstrated a CIS of 3 or greater. Lameness was observed in 1 BVDV-group calf prior to inoculation on days -3 to 0, as well as on day 1 following inoculation, and data were included in all analyses. Lameness was observed in an additional 3 BVDV-group calves following inoculation. One calf demonstrated a 2/5 right hind limb lameness on day 6, which had resolved by day 7. One calf had a 2/5 right hind lameness on days 8 – 9, as well as day 20. Data from these 2 calves were retained in all analyses. The third BVDV-group calf (ID: 1177) had a 2-3/5 right hind limb lameness starting on day 8 and continuing on through study day 21. Due to the severity of a skin lesion associated with placement of the accelerometer and which did not respond favorably to additional padding, the accelerometer was removed on study day 21 from this calf. Data from this calf was removed because of predetermined exclusion criteria. Although simple in their application and well tolerated by most calves, the accelerometers did result in significant skin lesions in 3 calves.

Physical examination and clinicopathological findings—A significant interaction ($P < 0.01$) was identified between treatment group and trial day for rectal temperature. In BVDV calves, significantly greater rectal temperatures were detected on days 7 and 28 as compared to controls ($P = 0.002$). Total WBCs and neutrophil counts were significantly lower in BVDV calves on days 7 and 14 ($P \leq 0.035$) (Figures 2 and 3). A significant decrease in lymphocyte count was observed in BVDV group calves on day 7 ($P = 0.012$) (Figure 4). Haptoglobin, fibrinogen, and serum iron concentrations were not significantly different between groups following virus inoculation.

Virus isolation and virus neutralization data—All calves were confirmed negative on virus isolation and virus neutralization on day 0. All sham inoculated calves were negative on virus isolation throughout the study period. Virus isolation in BVDV inoculated calves

demonstrated positive results in 10/10, 4/10, and 1/10 animals on day 7, 14, and 21, respectively. None of the sham inoculated calves seroconverted based on virus neutralization assays performed on day 28. All BVDV inoculated calves (10/10) demonstrated virus neutralizing antibodies on day 28 of the study.

Behavior data—Behavioral data from one BVDV-group calf (ID: 1132) was removed due to baseline standing and walking behavior being greater than 3 SD from the BVDV-group mean for these respective baseline standing behaviors. Behavioral data from two control-group calves (ID: 1105; 1178) were removed due to baseline walking behavior being greater than 3 SD from the control-group mean for baseline walking behavior. Data for the other behaviors from these 3 calves were included in the other analyses. Data from calves exhibiting lameness were included in all analyses if behaviors were within 3 SD from group means for those respective behaviors on days in which lameness was identified. Only 1 BVDV-group calf (ID: 1177) met criteria for exclusion, and behavioral data for time spent standing, lying, and walking were removed from study days 8-21. In this particular calf, the accelerometer was removed on study day 21 because of severity of the lesion associated with the accelerometer, and lack of response to additional padding.

Overall trends of behavior change observed in BVDV calves following inoculation consisted of a decrease in time spent walking and an increase in time spent lying compared to baseline. Following BVDV inoculation, increases or decreases in time spent standing compared to baseline behavior were observed and varied by study day. A significant interaction ($P < 0.01$) was identified between treatment group and trial day for time spent lying, time spent walking, and time spent standing. Significant increases in time spent lying in BVDV calves were observed on days 3 and 8 ($P = 0.037$ and 0.003, respectively) (Figure 5). BVDV calves spent significantly

less time walking on days 8 and 9 (P = 0.025 and 0.013, respectively) compared to baseline (Figure 6). On day 8, BVDV calves spent significantly less time standing (P = 0.001) compared to baseline, whereas significantly increased time standing was observed on days 14, 26, and 27 (P = 0.026, 0.023, and 0.026, respectively) (Figure 7). Following sham-inoculation, significant changes in behavior in control calves included decreased time spent standing on days 4 and 15 (P = 0.005 and 0.027; Figure 7), decreased time spent walking on days 8 – 12 (P <0.02; Figure 6), and increased time spent lying down on days 1, 4, 5 and 15 (P <0.02; Figure 5) compared to baseline behavior. Comparing changes from baseline behavior between groups, the time spent standing was significantly different on day 8, with BVDV calves spending significantly less time standing than controls (P = 0.001; Figure 7).

Discussion

The main objective of the present study was to assess the capabilities of remote behavioral data collection using accelerometers in cattle in a subclinical disease model. Use of the low-virulent BVDV 2 strain 134F resulted in the development of very subtle clinical illness, mild changes on clinicopathological measures, and detectable sickness behavior in BVDV-challenged calves. For all BVDV-group calves prior to inoculation, clinical illness scores and degree of activity were as expected for healthy calves, and all study calves were virus isolation and virus neutralization negative on day 0. Onset of clinical disease, as determined by subjective assessment, was seen during viremia in some but not all BVDV calves. Mild signs of depression suggest a mild acute phase response was successfully induced in BVDV-challenged calves. As expected, based on previous studies, this time period corresponds to the period of viremia following acute BVDV infection. Further, 10/10, 4/10, and 1/10 BVDV calves were virus isolation positive on days 7, 14, and 21, respectively. Clinical illness scores were normal

(CIS=1) for calves demonstrating a viremia on days 14 and 21. In moderate to severe acute BVDV infection models, onset of transient pyrexia occurs at approximately 48-72 hours post-exposure followed by persistent and marked pyrexia at 6-8 days with concurrent clinical signs of depression, anorexia, weakness, and diarrhea.[563,569] All control calves had CIS of 1 during this time period and remained negative on virus isolation testing during the 35-day study and did not seroconvert. Use of clinical illness scores, as previously supported in other studies[462,572], lacks sensitivity for the detection of disease in cattle. Although no statistical analysis was carried out on CISs due to a lack of blinding of the human observer to treatment groups, the lack of detection of mild clinical disease by subjective assessments in the present study would support the conclusions from previous studies.[177,462] However, in order to minimize human interference with changes in sickness behavior, limited clinical assessment of calves was carried out once daily, which could have decreased the ability of human observation to readily detect subtle changes in CISs in either BVDV or control calves.

As predicted in acute BVDV infection, BVDV calves had significantly greater rectal temperatures along with significantly lower total WBCs and neutrophils following inoculation. Although less severe, as a mild strain of BVDV was utilized in the present study, these findings are in agreement with WBC changes reported on days 6 – 12 post inoculation[569], and as early as day 3 – 12.[563] No significant differences were found between groups following inoculation in serum iron and the acute phase proteins haptoglobin and fibrinogen. This is in contrast to the report by Ganheim et al. (2003) where increased concentrations of haptoglobin starting 4-8 days post-inoculation, peak levels at 8.5 – 9.5 days, and a return to levels below detection by 13 days post-inoculation was observed in calves experimentally inoculated with BVDV.[177] Furthermore, fibrinogen concentration reached maximum levels by days 8 – 9 and returned to baseline by day

15.[177] The present findings are similar to those of Burciaga-Robles et al. (2010), in which exposure to BVDV alone did not result in increased haptoglobin concentrations.[188] While the mild nature of the present challenge model may not have been sufficient to stimulate production of haptoglobin and fibrinogen, significant differences in leukocyte count would argue against the idea of an undetectable acute phase response. It is more likely the timing of sampling at 7 and 14 days post challenge did not correspond to either an increase in concentrations or return to baseline concentrations of the acute phase proteins. The use of acute phase proteins did not prove useful in demonstrating morbidity in the present subclinical disease model. Continuous data collection technologies, such as accelerometers utilized in the present study, may be more appropriate in the detection of changes in behavior associated with disease. Potentially these technologies will allow for the prediction of disease onset, particularly when disease is subclinical or very mild in its degree of severity. Continuously monitoring behavior removes the need to predict appropriate sampling times and handle cattle for invasive, costly procedures. Use of these technologies may help to addresses the lack of sensitivity in correctly identifying morbid animals by human observation alone.

To the authors' knowledge, the present study is the first in which behavior changes following acute BVDV infection have been characterized using remote, objective behavior monitoring technology. Our findings are in agreement with previous experimental challenge studies utilizing remote monitoring technologies indicating that the devices can detect changes in behavior following exposure. Hanzlicek et al. (2010) demonstrated significantly lower mean step counts and behavioral changes in beef steers inoculated with *Mannheimia hemolytica* compared to controls. Cattle spent a greater percentage of time standing than lying prior to the induction of pneumonia, whereas 4 days post-inoculation, cattle spent a significantly greater percentage of

time lying than standing.[462] The data in the present study would also indicate cattle spend greater percentage of time lying than standing following a low-virulent BVDV challenge. Although the use of pedometers and accelerometers did not demonstrate a correlation between the degree and severity of disease progression in the study, data provided evidence that behavioral changes associated with respiratory disease were detectable via accelerometers.[462] In another study using a remote location monitoring device, sickness behaviors were demonstrated in calves intranasally inoculated with *Mycoplasma bovis*.[411] By mapping specific areas of interest in the pen (waterer, feed bunk, and shelter), time spent by each calf at respective locations as well as the total distance traveled per day were objectively quantified. Clinical illness scores were associated with time spent at specific locations in the pen, as well as the distance traveled.[411] Alteration of behavior was significantly different in calves with more severe disease compared to those with mild disease, and tended to reflect the degree of lung pathology.[411] The results from the present study augment previous findings that accelerometers are useful in the detection of changes in cattle behavior. Changes in behavior from baseline were observed in both the control and BVDV calves, albeit at different time points following sham or BVDV inoculation. Overall trends in time spent lying, standing, and walking were similar for both control and BVDV calves. The use of BVDV 2 strain 134F was expected to induce very mild disease, which likely limited the ability to demonstrate significant differences in behavior between BVDV and control calves. Potential future studies should utilize more virulent strains of BVDV in order to refine the detection capabilities of accelerometers in BVDV-disease models. Difficulty in demonstrating contrast in behavior changes between BVDV and control calves was compounded further by the necessity to house BVDV and sham-inoculated calves separately in biosecure pastures due to the infectious nature of BVDV. This undoubtedly contributed to the pen effect observed. Although

the topography and acreage of each pasture was roughly equal, pastures were not identical and may have been a confounding variable in behavior differences observed between BVDV and control calves. However, significant difference between the time spent standing between groups was observed on day 8 following inoculation. Decreased standing and walking behavior in the BVDV group during the time of viremia was demonstrated. Use of accelerometers may allow detection of behavior changes in acute BVDV infection.

Unfortunately, lameness due to accelerometer placement was observed in 3 BVDV calves, potentially influencing the findings as significant lameness was present in 1 BVDV calf during the baseline activity acquisition period and in 1 BVDV calf during viremia. Extreme variation from group cohorts was found in only 1 of the calves demonstrating lameness. This data was excluded from analyses. Otherwise, data from the other calves were retained. From a practical standpoint and the potential use of such devices in the commercial setting, changes need to be made to the fastening mechanism of this specific accelerometer model for long term data acquisition in light weight cattle under environmental conditions of relatively high humidity and ambient temperature, as in the present study. Undoubtedly lameness will alter the animal's behavior and limit the application of this technology[498], unless a more ergonomic model is utilized. Such models are commercially available, albeit with different data acquisition capabilities, that have proven well tolerated in both adult cattle and young calves in on-going studies by the authors.

The present report demonstrates the potential usefulness and sensitivity of accelerometers for remote monitoring of sickness behavior in calves in a subclinical BVDV model. The utility of objective remote monitoring in real-time may be of more use in disease detection compared to

more traditional markers on inflammation, including acute phase proteins and subjectively-based clinical illness scores.

Footnotes

a. Gibco Life Technologies Corp., Grand Island, NY.

b. Hyclone, GE Healthcare Life Sciences, Logan Utah.

c. GP1 Programmable Accelerometer, Sensr Company, Elkander, IA

d. Insightful Miner, Insightful Corporation, Seattle, WA

e. Phase Haptoglobin Assay, Tridelta Development Ltd., Maynooth, CO Kildare, Ireland

f. G*Power 3.1.9.2, Heinrich-Heine-University, Dusseldorf, Germany

g. PROC GLIMMIX, SAS, version 9.2, SAS Institute Inc, Cary, NC

Figure 2. Mean ± SE Total white blood cell concentration (x10^3/uL) in BVDV (n = 10, striped bars) and control (n = 10, solid bars) calves experimentally infected with a low-virulent strain of BVDV immediately before (day 0) and at 7-day intervals after inoculation. Calves in the BVDV group were inoculated with 2mL of noncytopathic BVDV type 2 strain 134F (10^6 $TCID_{50}$/mL) in each nostril, and calves in the control group were sham inoculated with 2mL of BVDV-free medium in each nostril.

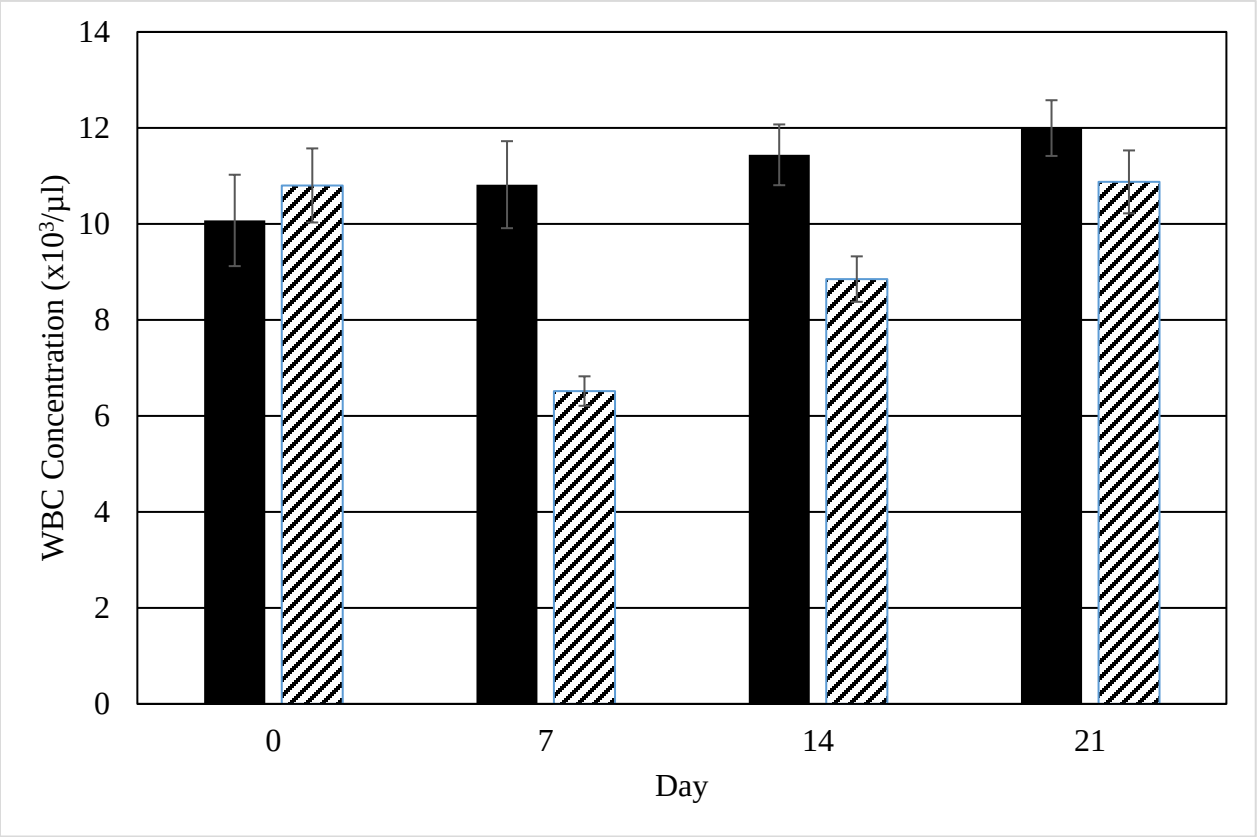


Figure 3. Mean ± SE Neutrophil concentration ($x10^3$/uL) in BVDV (n = 10, striped bars) and control (n = 10, solid bars) calves experimentally infected with a low-virulent strain of BVDV immediately before (day 0) and at 7-day intervals after inoculation. Calves in the BVDV group were inoculated with 2mL of noncytopathic BVDV type 2 strain 134F (10^6 $TCID_{50}$/mL) in each nostril, and calves in the control group were sham inoculated with 2mL of BVDV-free medium in each nostril.

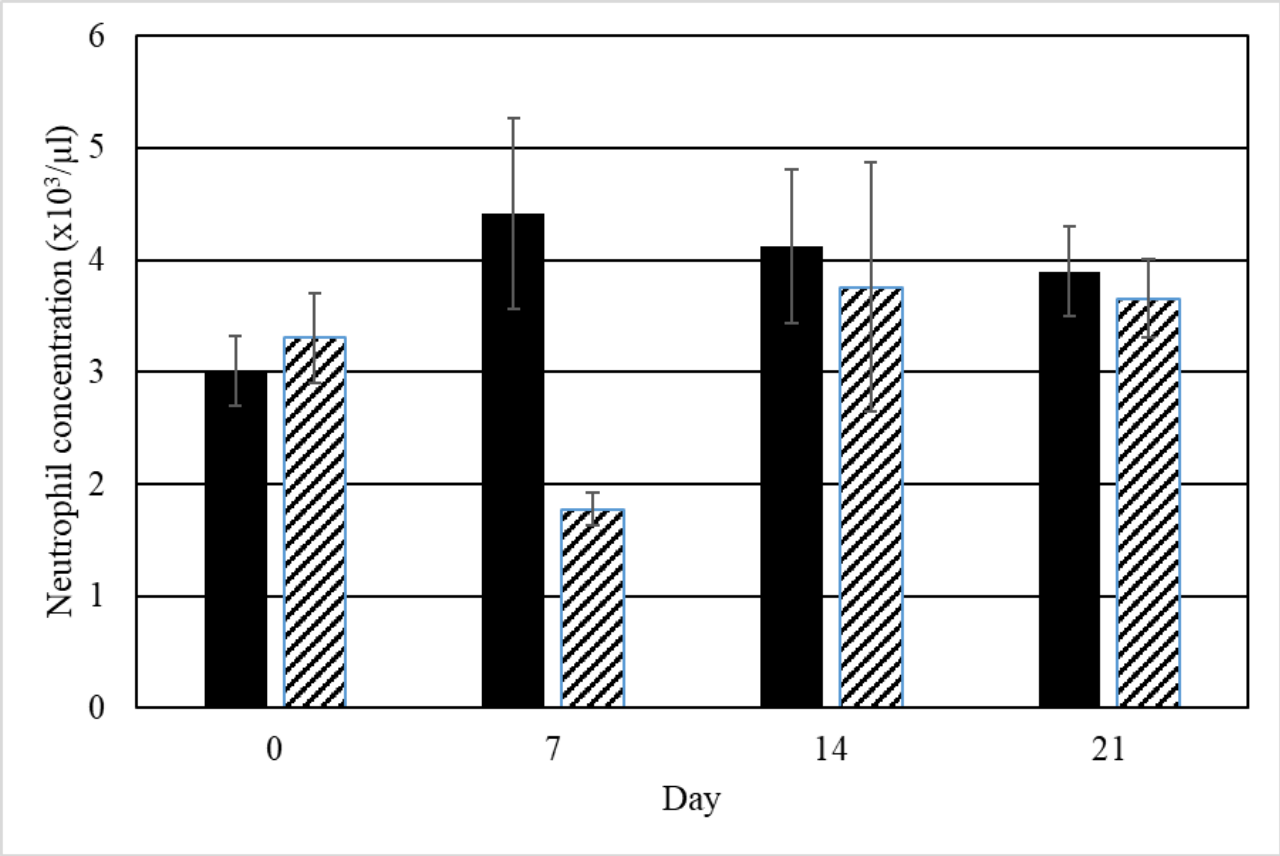


Figure 4. Mean ± SE Lymphocyte concentration ($x10^3$/uL) in BVDV (n = 10, striped bars) and control (n = 10, solid bars) calves experimentally infected with a low-virulent strain of BVDV immediately before (day 0) and at 7-day intervals after inoculation. Calves in the BVDV group were inoculated with 2mL of noncytopathic BVDV type 2 strain 134F (10^6 $TCID_{50}$/mL) in each nostril, and calves in the control group were sham inoculated with 2mL of BVDV-free medium in each nostril.

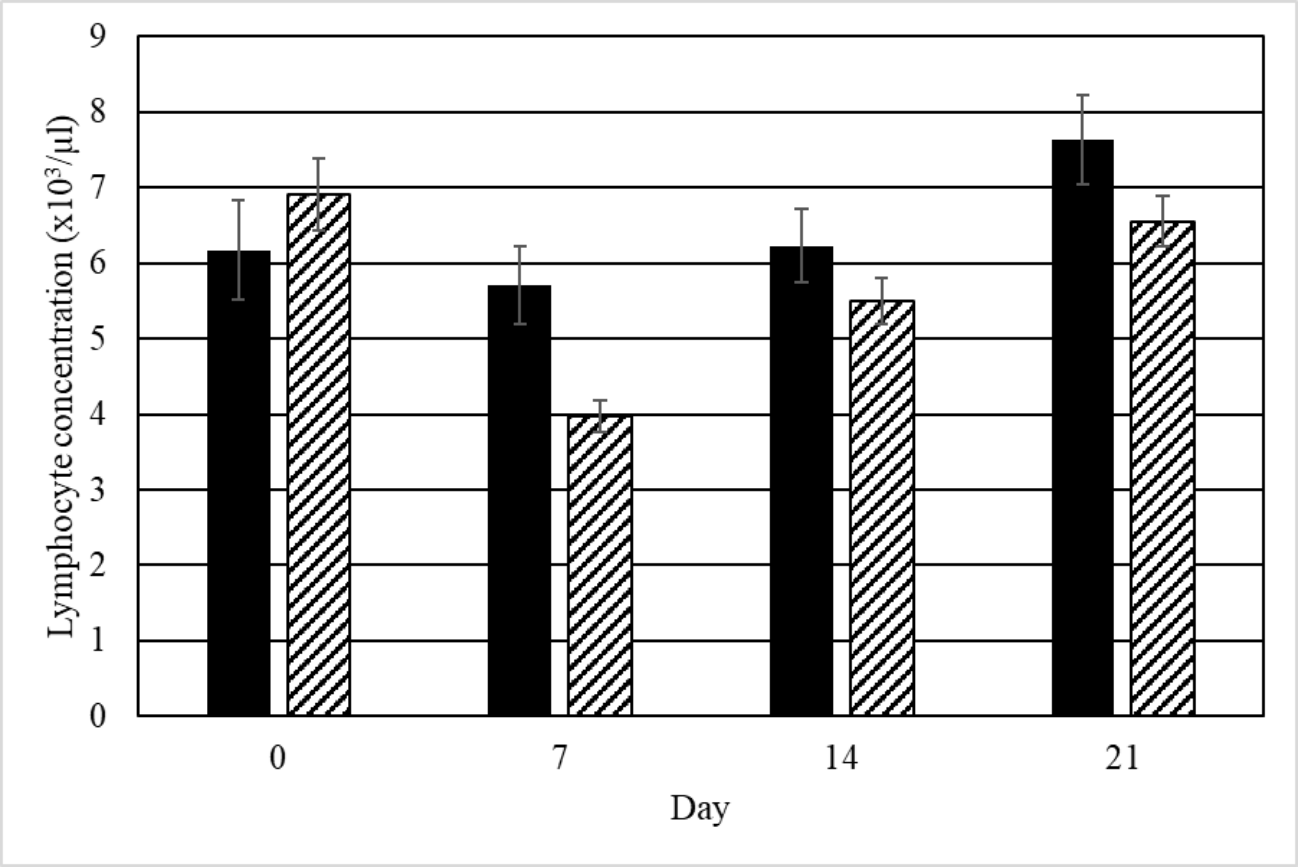


Figure 5. Ratio of the mean time spent lying after experimental inoculation (day 0) to the mean time spent lying during the 7 days before experimental inoculation (days -7 to -1; baseline) for the calves of the BVDV (dotted line) and control (solid line) groups. The brackets delimit the range for each ratio on a given day. The accelerometer was removed from 1 calf in the BVDV group on day 21 because of skin lesions and lameness, and the behavioral data from that calf were excluded from all analyses. Therefore, the values for the BVDV group represent the means for 9 calves. [a]Within a day, the mean time spent on the given behavior differs significantly ($p < 0.05$) from baseline for the control group. [b]Within a day, the mean time spent on the given behavior differs significantly ($p < 0.05$) from baseline for the BVDV group. [c]Within a day, the ratio for the BVDV groups differs significantly ($p < 0.05$) from that for the control group.

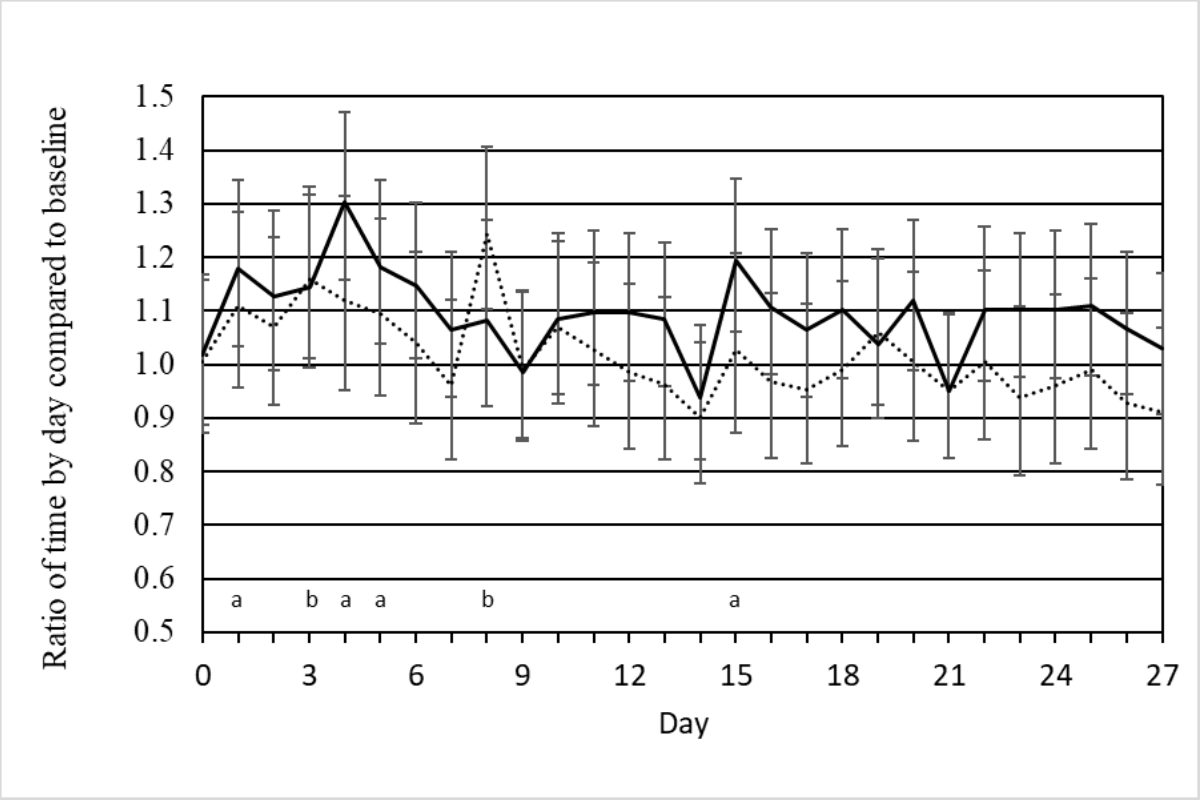


Figure 6. Ratio of the mean time spent walking after experimental inoculation (day 0) to the mean time spent walking during the 7 days before experimental inoculation (days -7 to -1; baseline) for the calves of the BVDV (dotted line) and control (solid line) groups. The brackets delimit the range for each ratio on a given day. The accelerometer was removed from 1 calf in the BVDV group on day 21 because of skin lesions and lameness, and the behavioral data from that calf were excluded from all analyses. Therefore, the values for the BVDV group represent the means for 9 calves. [a]Within a day, the mean time spent on the given behavior differs significantly ($p < 0.05$) from baseline for the control group. [b]Within a day, the mean time spent on the given behavior differs significantly ($p < 0.05$) from baseline for the BVDV group. [c]Within a day, the ratio for the BVDV groups differs significantly ($p < 0.05$) from that for the control group.

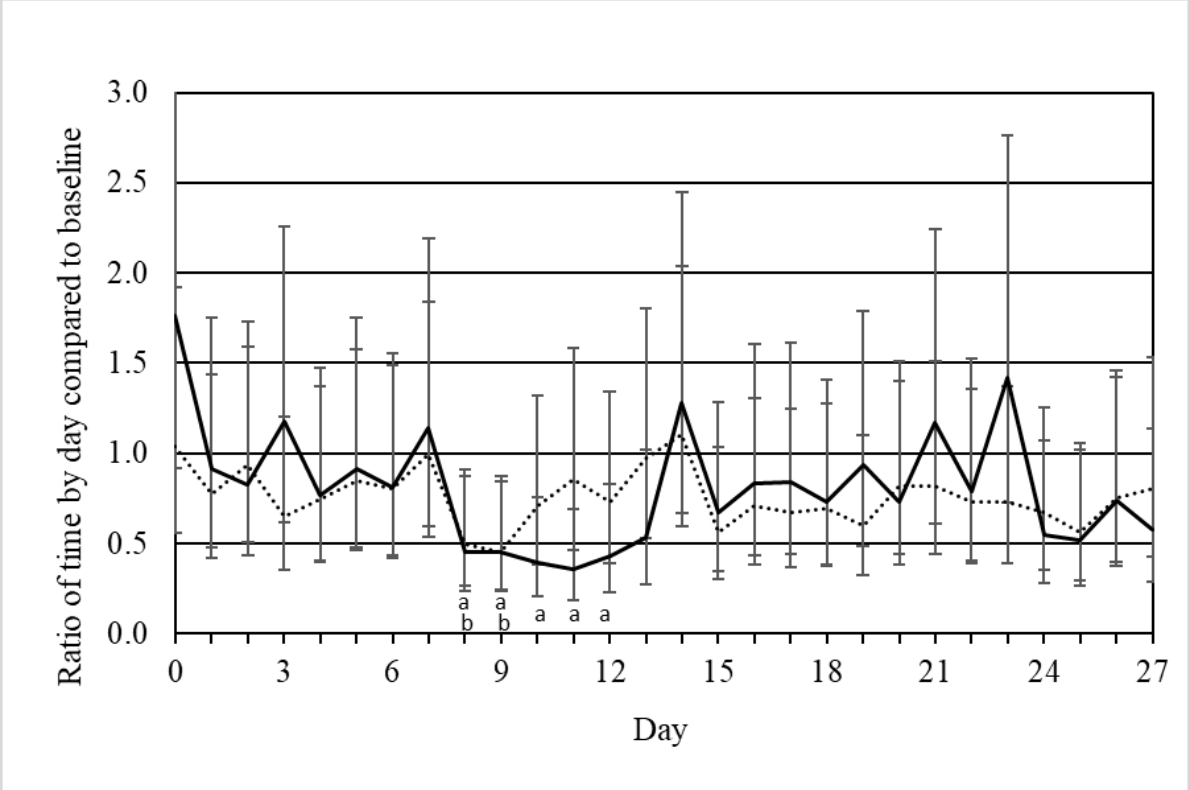
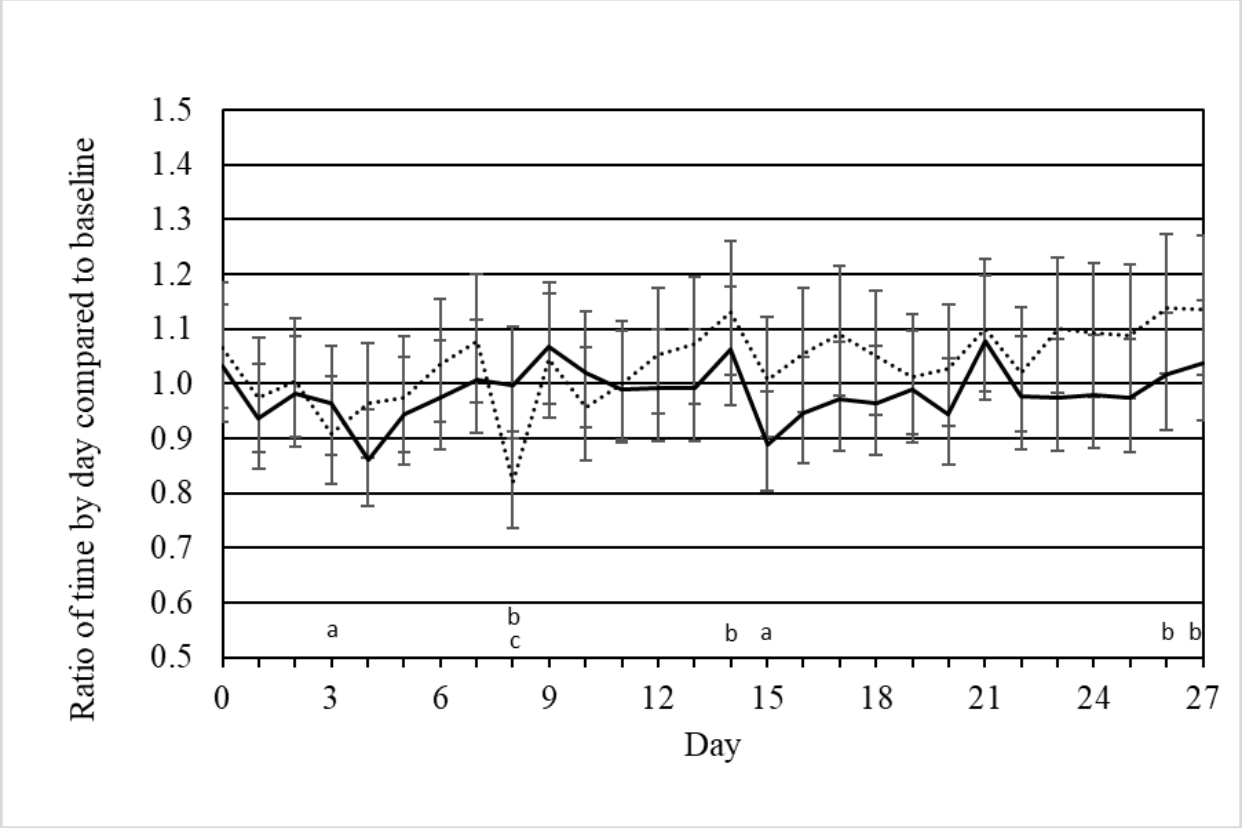


Figure 7. Ratio of the mean time spent standing after experimental inoculation (day 0) to the mean time spent standing during the 7 days before experimental inoculation (days -7 to -1; baseline) for the calves of the BVDV (dotted line) and control (solid line) groups. The brackets delimit the range for each ratio on a given day. The accelerometer was removed from 1 calf in the BVDV group on day 21 because of skin lesions and lameness, and the behavioral data from that calf were excluded from all analyses. Therefore, the values for the BVDV group represent the means for 9 calves. [a]Within a day, the mean time spent on the given behavior differs significantly ($p < 0.05$) from baseline for the control group. [b]Within a day, the mean time spent on the given behavior differs significantly ($p < 0.05$) from baseline for the BVDV group. [c]Within a day, the ratio for the BVDV groups differs significantly ($p < 0.05$) from that for the control group.



Chapter 4: Manuscript: Evaluation of periparturient behavior in beef cattle using three-dimensional accelerometers

Jenna E. Bayne, DVM, DACVIM[1], Paul H. Walz, DVM, PhD, DACVIM[1], Thomas Passler, DVM, PhD, DACVIM[1]

[1] College of Veterinary Medicine, Departments of Clinical Sciences and Pathobiology, Auburn University, Auburn, AL 36849, USA

Supported by American Association of Bovine Practitioners and Auburn University Interdisciplinary Research and Equipment Grants Program.

This manuscript represents a portion of the dissertation submitted by Dr. Bayne to the Auburn University Graduate School as partial fulfillment of the requirements for Doctor of Philosophy degree.

Presented in part at the American Association of Bovine Practitioners Annual Conference, New Orleans, LA, September 2015.

Introduction

On beef cow-calf operations, adequate calving management is important to prevent cow and calf morbidity and mortality due to adverse events during the periparturient period. Sustainable beef production requires the delivery of healthy, live calves and retention of dams with sound breeding efficiency.[573] The impact of dystocia and related conditions caused by prolonged birth is well documented[573-576], and dystocia constitutes the leading cause of death of beef calves.[574,575,577] Reported dystocia rates in beef cattle for which intervention was eventually required range from less than 5% to over 18%.[573,575,576,578,579] Although the vast majority of cows and heifers typically do not require assistance during calving,[580], the reproductive efficiency and the overall longevity of cows is significantly impacted by several calving management practices, which include appropriate frequency of observation and timely intervention.[573] Recent survey data indicate that improvement in reproductive efficiency is warranted.[580] Approximately 50% of cow-calf operations have a defined breeding season and most, but not all, producers regularly observe their animals during the calving season.[580] According to a recent survey of U.S. cow-calf producers, heifers were observed 3.6 times per 24 h compared to 2.5 times per 24 h during the calving season for cows; however, 9.0% of operations reported observing cows less than once per day.[573] Intervention was offered following 2.8 h and 3.5 h of labor on average in heifers and cows, respectively, and assistance was required in 16.7% of heifers and 2.7% of cows.[573] Cows that experience prolonged and difficult calvings can develop dystocia-related problems including retained fetal membranes and calf loss early in the post-partum period.[574,581] These cows have lower pregnancy rates in the subsequent breeding season[581] and are at an increased risk of

culling.[582] Independent of breed, longevity is significantly impacted in cows that require intervention or have stillbirths.[583]

Identification of impending parturition and the onset of calving have conventionally relied upon timely and frequent observation by experienced stockmen. External visual clues include enlargement of the udder, relaxation of the pelvic ligaments and perineum, tumefaction of the vulva, and vaginal mucus discharge. Changes in behavior include isolation from herd mates, nest-building, signs of abdominal discomfort, and a reduction in feed intake.[584-587] During eutocic births, parturition progresses through three sequential stages. The first stage of labor is characterized by relaxation and dilation of the cervix, with a duration of approximately 6 h, but up to 24 h in heifers[588] and ends with rupture of the chorioallantois. The second stage of labor encompasses the delivery of the calf. During this stage, cows demonstrate frequent bouts of lying and standing as abdominal contractions increase in frequency and intensity.[442,443] Cows typically lie down in sternal and eventually lateral recumbency as stage two nears completion, for expulsion of the calf. The average length of stage 2 labor in pluriparous cows is approximately 2 to 4 h, but can be longer in primiparous dams.[587,589] Following calving, the cow stands and engages in licking and stimulating the calf to rise and nurse. During this third stage of labor, expulsion of the fetal membranes occurs, on average by 8 h, signifying the completion of labor.[590]

Progressive changes in the relaxation of the sacrosciatic ligament has been demonstrated to be predictive of calving within 24 h, with reported accuracy ranging from 82%[588] to 94%.[591] Changes in the placental estrogen unconjugated β-estradiol correlated well with relaxation of the sacrosciatic ligament and was also found to be predictive of calving within 24 h, with an accuracy of 85.2%.[591] However, the onset and progression of external changes such as udder

development and vulvar appearance can vary greatly between individual animals and can even be misleading in predicting an accurate calving date.[588] Blood progesterone concentrations dramatically drop to about 1 ng/ml or lower approximately 24 h before calving.[592] Use of a commercially available cow-side EIA demonstrated a sensitivity, specificity, positive predictive value, and negative predictive value of 80%, 97.6%, 88.9%, and 95.3%, respectively, for the prediction of calving within 24 h when progesterone levels drop below 2 ng/ml.[592] When combined with a parturition scoring system based on external cues, blood progesterone concentrations had a 96.8% probably of ruling out calving within 24 h but a relatively low ability to predict parturition within 12 h, at 53%.[593] Prediction of calving based on observations requiring repeated close contact with the handler, restraint of the animal, or repeated sampling is labor intensive, invasive, and attainable only in housed dairy cattle. Use of these parameters to predict calving is unrealistic in pastured beef cattle.

The refinement of technologies capable of remotely capturing real-time, objective activity data may allow the use of cattle behavior patterns and changes in physiological parameters to predict the onset of calving. Application of technologies, which could identify dams experiencing dystocia and therefore facilitate more timely human intervention, has the potential to improve calf survivability and reproductive efficiency in beef herds. Examples include continuous monitoring of vaginal and ruminal temperatures[293,307,594], feed intake and rumination activity[307,396-398,400,401,595,596], tail carriage[597,598], and video surveillance of cattle behaviors.[439-441] Measureable changes in cow activity behaviors such as time spent lying, steps taken, or lying bout frequency during the peripartum period were demonstrated by the use of accelerometers and showed promise in predicting the onset of calving in housed dairy cattle.[440-443,589] The objectives of the present study were to assess behavioral activity patterns using

accelerometers in pastured beef cows and heifers during the peripartum period and to determine if changes in activity indices were predictive of calving.

Materials and Methods

All study procedures were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2013-2366). The study was conducted at the Auburn University NABU over two consecutive fall-calving seasons. Forty multiparous crossbred beef cows (first calving season, first year of study, October – December; MP) and forty primiparous crossbred beef heifers (second calving season, second year of study, August – November; PR) were enrolled in the study. Animals were maintained as one group in the respective seasons on an 8-ha biosecure pasture throughout the study period. For all animals, water and Bermuda grass hay were provided ad libitum, and a 12% protein concentrate was fed once daily at a fixed time (8:00AM) to allow for distant visual assessment of each animal and correct positioning of accelerometers. A minimum of 60 d prior to the anticipated start date of calving, each dam was confirmed pregnant by rectal palpation and had a commercially manufactured accelerometer (IceQube, IceRobotics™) attached to the lateral aspect of the right hind limb just proximal to the metacarpophalangeal joint, as previously described[414], for continuous activity monitoring throughout the study period. The pasture was evaluated every 8 hours daily for cows separated from the group and for presence of newborn calves throughout the calving seasons. Newborn calves were weighed and identified with an ear tag immediately upon finding during pasture evaluations. However, parturition was intended to be unobserved, as to minimize interference of human observation on calving behavior. Therefore, the exact time of calving was undetermined. Most calves had been cleaned off by the dam and had nursed colostrum prior to initial handling. The date and time were recorded for each calving (when calf found) for each cow-calf pair

throughout the study. Accelerometers were removed on d 7 post-calving and data were downloaded. For animals that did not calve within 60 d of device placement, the device was temporarily removed, data downloaded, and the accelerometer reapplied for further data capture until d 7 post-calving.

The data continuously collected by the device were reported in 60-minute intervals. Quantification of activity indices included time spent standing, lying, and walking for each time block and the number of times cows transitioned from standing to lying positions (lying bouts) per time block. The final 24 h prior to calving was assigned day -1 and the immediate 24 h post-calving assigned day 1, which included time 0. Time of parturition, assigned time 0, was unobserved but was approximated for each cow based on the marked increase in lying bout frequency, based on visual evaluation of individual animal data graphically displayed (results not shown), as described in previous studies.[442,443] The total observational period included 14 d pre-calving (d -14 to -1) and 7 d post-calving (d 1 to 7).

Statistical analysis

Data analyzed included only animals that calved naturally, without assistance and had a viable calf for 7 d post-partum. Activity data collected during a defined 21-d observation period, which included 14 d pre-calving and 7 d post-calving were analyzed. Throughout the observation period, the activity of each animal was categorized as lying, walking, standing, number of lying bouts, and number of steps taken, based on data obtained from the accelerometer. Data represented (or were captured as) 60-minute blocks, and each day of the observation period was comprised of 24 1h blocks. The data were assessed for normality by visual inspection of frequency distribution and by the Shapiro-Wilk test as implemented in JMP 11.0.0 (SAS Institute, Cary, NC). Activity indices of standing time, lying time, steps, and lying bouts were

nonparametric in nature. For each day, the median time each animal spent standing and lying were calculated. Similarly, medians for lying bouts and steps taken were calculated. Comparison of each behavior by day over the 21-d observational period were made using a Friedman test as implemented in Prism 7 (GraphPad Software, San Diego, CA).

Results

The duration of the first calving season was from October 18 until December 13 for the multiparous cows. Data analyzed included thirty-seven of the initially enrolled forty multiparous cows during the first calving season. Of the three cows for which data were excluded from analyses, two cows delivered stillborn calves and the third hid a viable calf, which could not be found within 24 h of birth. The duration of the second calving season was from September 1 until October 13 for the primiparous heifers. Analyzed data included thirty-three of the forty initially enrolled primiparous heifers during the second calving season. Dystocia occurred in four primiparous heifers, resulting in assisted delivery of two viable and two dead calves. Additionally, two unassisted stillbirths and the mis-mothering of a single calf occurred. Therefore, the data from these seven animals were excluded from analyses.

Graphic representation of behavioral activity indices standing time, lying time, steps, and lying bouts are shown in Figures 8 – 11, respectively. The average standing time per day was similar during d -14 to -2. A significant increase in standing time was observed on d -1 compared to previous activity ($P < 0.0001$). Standing time continued to increase on d 1 compared to pre-calving standing time ($P < 0.02$), with a gradual reduction in standing time observed on d 2 – 7 post-calving. However, standing time post-calving continued to remain above pre-calving levels for the remainder of the observational period. Based on 8-h period data analysis, a significant increase in the median standing time was observed as early as 24 to 16 h prior to calving and was

significantly different from median standing times post-calving ($P < 0.01$). Mean lying time per day was similar during d -14 to -2. A significant decrease in lying time was observed on d -1 compared to previous activity ($P < 0.03$). Lying time continued to decrease on d 1 compared to pre-calving time ($P < 0.02$), followed by a gradual increase in lying time on d 2 – 7 post-calving, with return to baseline lying time by d 7. Based on 24-h period data analysis, median lying times were significantly different at 48 h prior to calving compared to baseline and continued until d 2 post-calving ($P < 0.025$). However, no significant differences in lying time were found between 8-h period data. Average steps taken per day were similar on d -14 to -2 pre-calving. A marked increase in steps counts was observed on d -1, followed by a marked decrease below baseline levels as of d 1 post-calving ($P < 0.0001$). For the remainder of the observation period, step counts on d 2 – 7 remained significantly below prepartum levels ($P < 0.01$). Based on 24-h period data analysis, median steps taken on d -2 were not significantly different from d -1, whereas median steps on d -1 were significantly different from d 1 – 7 post-calving ($P < 0.0001$). Based on 8-h period analysis, significant differences in median steps taken per day were observed within 24 to 16 h of calving and remained significantly different from median steps on d 1 – 7 post-calving ($P < 0.01$). Given the predetermined definition of calving (time 0), lying bouts significantly increased from baseline on d -1 ($P < 0.0001$). Following parturition, lying bouts gradually decreased and returned to pre-calving values by d 3 ($P < 0.01$; Figure 11). Median lying bouts on d -14 through d -2 significantly differed from median lying bouts on d -1 and d 1 ($P < 0.0001$). The 24–h-period prior to calving significantly differed in median lying bouts on d 2 – 7 post-calving ($P < 0.0001$). Median lying bouts were not significantly different for d -1 compared to d 1. Based on 8-h period analysis, significant differences in median lying bouts could be demonstrated as early as 24 to 16 h prior to calving ($P < 0.0001$).

Discussion

Several types of remote monitoring technologies to objectively measure physiological and behavioral changes in both beef and dairy cattle have been evaluated in an effort to improve animal health and productivity. To date, there are commercial systems capable of capturing real-time data on multiple variables, including temperature, ruminating and eating behavior, and activity.[389] Assessment of body temperature using rumen boluses has demonstrated the ability to detect both morbidity associated with diseases, such as bovine viral diarrhea virus (BVDV) [300] and bovine respiratory disease (BRD)[301], as well as the detection of physiological events such as estrus or parturition.[307] Use of rumen temperature boluses in Angus beef cows demonstrated a significant decrease in rumen temperature 48 to 24 h before the onset of parturition.[307] In a small pilot study, continuous vaginal temperature monitoring was reported to be predictive of calving within 36-60 h once a decrease of ≥0.3°C was observed, with an accuracy of 74%.[293] In agreement, Burfeind et al. detected a gradual decrease in temperature starting 48 h prior to parturition, with a nadir reached approximately 12-18h before calving.[594] A change of 0.2°C to 0.3°C in vaginal or rectal temperature was demonstrated to have a predictive accuracy of 77-86% and 73-91%, respectively, for the occurrence of calving within the following 24 to 48 h.[594] Use of bunk and water attendance systems, which quantify feeding and drinking behavior, are capable of detecting morbidity associated with bovine respiratory disease prior to conventional methods in feedlot cattle.[361] Similarly, changes in prepartum dry matter intake (DMI), feeding time, and water intake were found predictive of post-partum diseases, such as metritis, hypocalcemia, and ketosis, weeks before the onset of clinical signs.[364,365,456,457] Rumination monitoring systems used as a standalone monitor or in conjunction with bunk attendance systems are simple in their application and difficulty of data collection. Studies demonstrated marked

reductions in rumination time, along with concurrent decreases in feeding time and DMI, to occur within 8 h and 1-2 d of calving, respectively.[396-400]

Initial efforts to characterize animal behavior by quantifiable, objective measures through remote monitoring that minimizes the impact of human observation on behavior have utilized video surveillance. More recently, use of accelerometers and other activity monitors have largely supplanted the use of video surveillance. These devices are capable of measuring static (e.g. standing, lying) and dynamic (e.g. walking) activities and have been validated to provide comparable evidence to that of direct observation.[409,414] Collectively, these remote technologies demonstrate changes in cattle behavior associated with changes in physiological states and the presence of disease. Using video surveillance under confined housing conditions, Barrier et al. detected that within 6 h of the onset of stage 2 labor, cows demonstrate an increase in restlessness, tail raising, abdominal contractions, changes in body posture and transitions from standing to lying.[439] Similar findings have been reported by Jensen[440] and Miedema et al.[441] However, the ability to distinguish between dystocic and normal calvings based on duration and intensity of these measures during stage 2 labor was limited, in part due to the large variability between individuals.[439,589] Dystocial cows were comparatively more restless, had their tail raised for longer and laid laterally with the head rested for longer.[439] The ability to distinguish dystocic from normal calvings, based on the magnitude and timing of increased lying bout frequency relative to stage 2 labor has been demonstrated in dairy cows by Proudfoot et al.[596], in contrast to the findings of others.[439,589] Use of video surveillance requires tedious and time-consuming review of data. Although computerized image analyses that fully automate this process have been developed[599], validation of these programs under field conditions and testing of the ability to distinguish normal from dystocic calvings has not been performed.

Characterization of behavioral changes using accelerometers and other activity monitoring devices have demonstrated comparable behavior changes in peripartum cows to the video surveillance data described above. In the present study, significant changes in behavior from baseline were observed, with increases in time standing, number of steps taken, number of lying bouts observed, and decreases in lying time observed. Marked changes were appreciable within 24 h of calving for all activity indices. This is in agreement with the previous findings of Huzzey et al. (2005), which demonstrated significant changes in mean daily standing time and an 80% increase in the number of transitions from lying to standing during 24 h prior to and 24 h post-calving period in free-stall housed transition dairy cows.[442] Based on accelerometer behavioral data in housed dairy cows, an algorithm to predict calving onset has recently been described by Titler et al.[443] Distinct behavioral patterns, which include an increase in step count, increased standing time, decreased lying time, and increased frequency of lying bouts, were similarly present in primiparous and multiparous dams.[443] Heifers spent less time standing and had fewer lying bouts compared to cows for the 24 h prior to calving, but both parity groups demonstrated similar changes in behavioral activity indices.[443] Using the activity index developed, which placed a greater significance on lying bout frequency compared to other behavior data, a significant change in activity was observed on average 6 h before calving (with a range of 2 to 14 h); and, in almost 76% of animals within 4 h of calving.[443] Overall, similar changes in behavioral indices were in the present study. However, in contrast to Titler et al.[443], heifers in this study spent more time standing and less time lying compared to cows. In addition, heifers had greater step counts throughout the observation period compared to cows. Prepartum lying bout frequency was slightly higher in heifers compared to cows, but the increase in lying bout frequency on d -1 was comparable between parity groups. However, direct comparison

between multiparous and primiparous dams is limited in this study, as the data represents successive and not concurrent calving seasons. Behavioral activity could be different between dam groups due to other extraneous factors. Although approximately the same acreage, different pastures were used for successive calving seasons, and weather conditions were variable.

Presently, only dams that calved naturally were included, with exclusion of dams having stillbirths or needing assistance during prolonged delivery. As parturition was unobserved, data from cows experiencing prolonged and difficult calvings, with delivery of a viable calf may have been included in the analyses. Although the present study has the limitation that calvings were not observed and time zero was approximated based on visual appraisal of individual cow lying bout frequency data, this experimental design was chosen to limit the influence of human interaction complicating the data sets. Overall, changes in behavioral activity were similar to those previously reported and that changes may be predictive of calving within 24-h. Further research involving the use of accelerometers and other activity monitoring technology in pastured beef cattle is needed in order to evaluate its potential to predict calving onset as well as identify abnormal behavioral activity indicative of dystocia.

Figure 8. Mean standing time by day. Data from cows and heifers combined, n = 70. Superscript letters indicate: [a] Standing time on day -1 differed from days -14 to -2 ($P < 0.0001$); [b] Standing time on day 1 differed from -14 to 7, excluding day -1 ($P < 0.02$). Superscript symbol indicate: [†] Median standing time on day -1 different from pre-calving baseline ($P < 0.01$). [±] Median standing time on day -1 different from d 2 – 7 ($P < 0.04$).

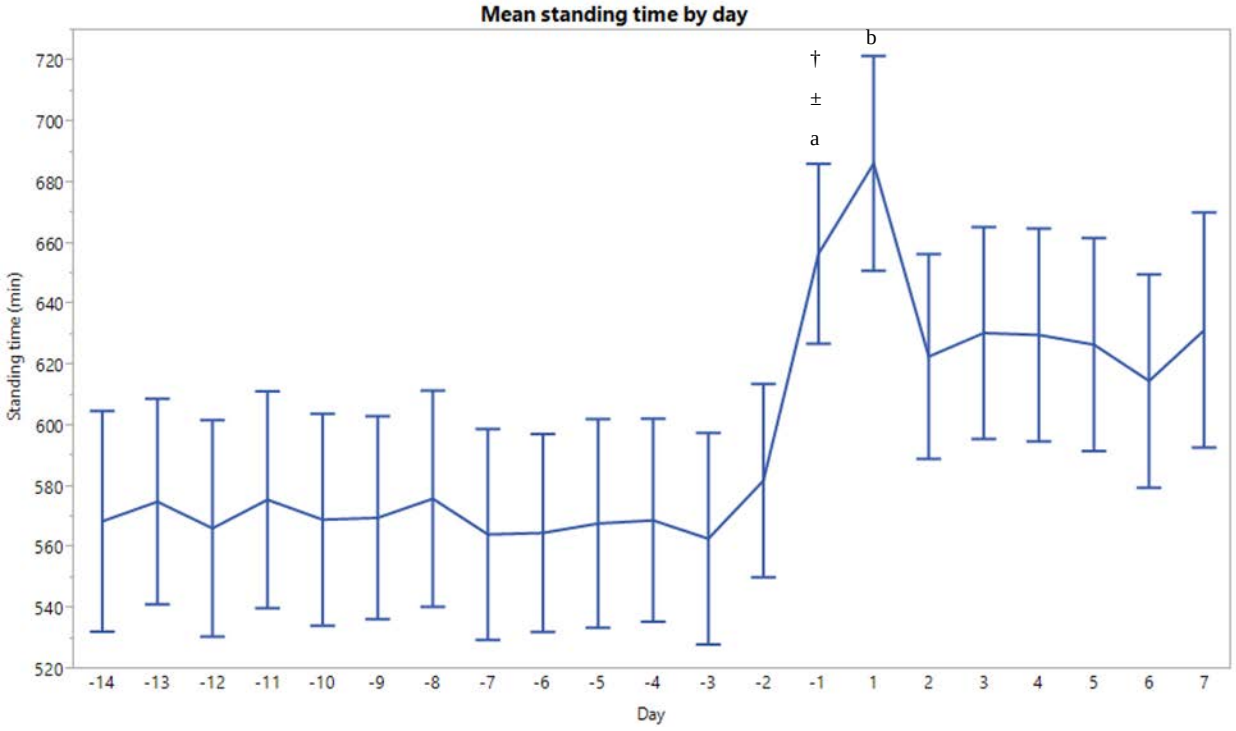


Figure 9. Mean lying time by day. Data from cows and heifers combined, n = 70. Superscript letters indicate: [a] Lying time on day -1 and 1 differed from days -14 to -2 ($P < 0.03$); [b] Standing time on day 1 differed from -14 to 7, excluding day -1 ($P < 0.02$). Superscript symbols indicate: [†] Median lying time on day -1 and 1 different from pre-calving baseline ($P < 0.025$). [±] Median lying time on day 1 different from d 2 – 7 ($P < 0.04$).

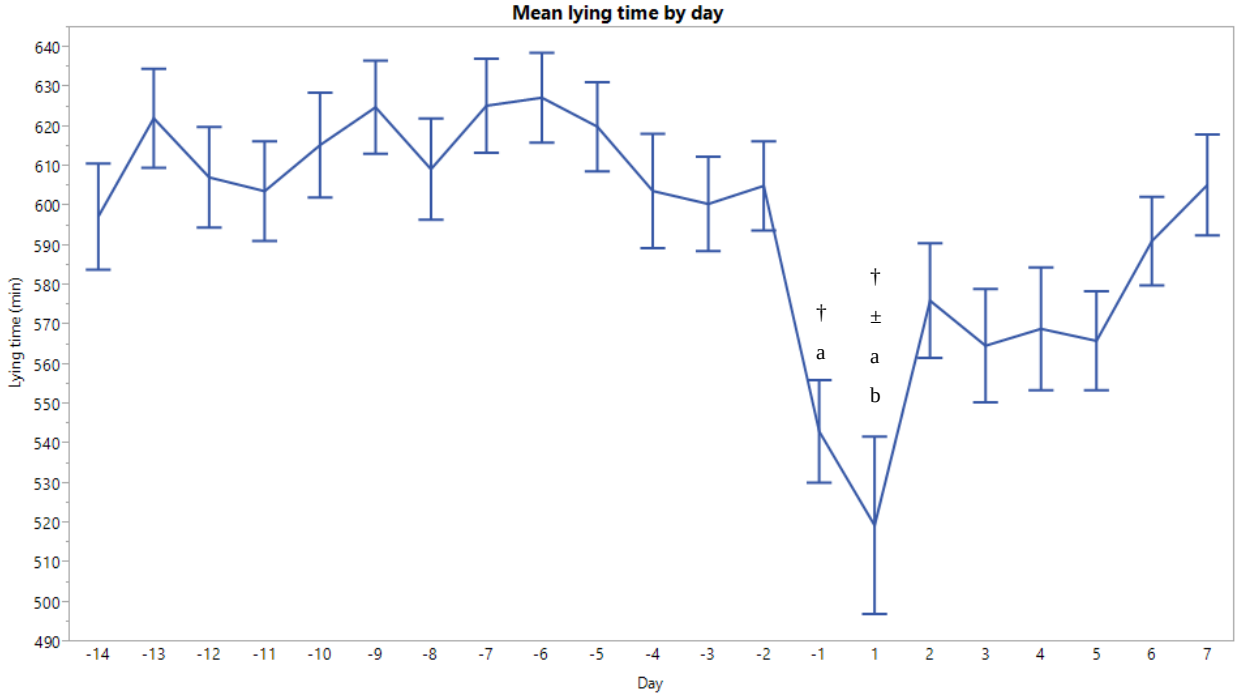


Figure 10. Mean steps by day. Data from cows and heifers combined, n = 70.

Superscript letters indicate: [a] Steps on day -1 differed from days -14 to -2 ($P < 0.0001$); [b] Day -1 differed from days 1 – 7. Superscript symbols indicate: [†] Median steps between -24 and -16 hours prepartum differed from day 1 – 7 ($P < 0.0001$). [±] Day -14 to -2 differed from day 1 – 7 ($P < 0.01$).

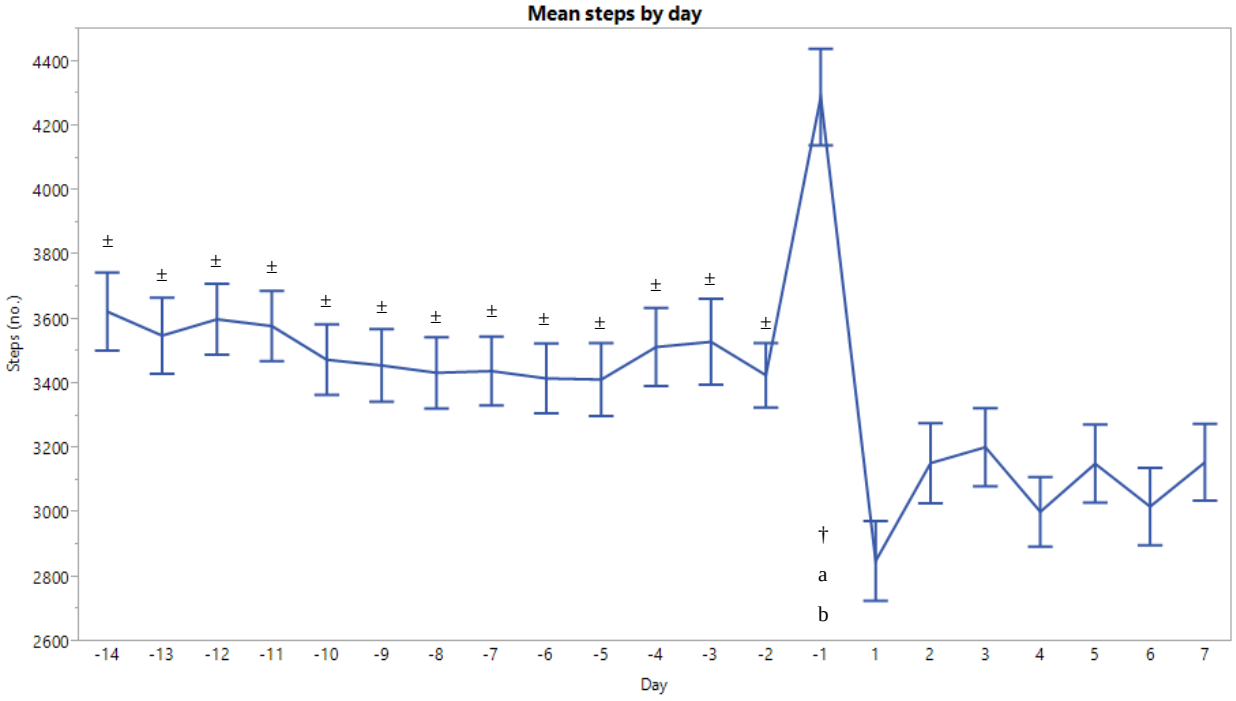
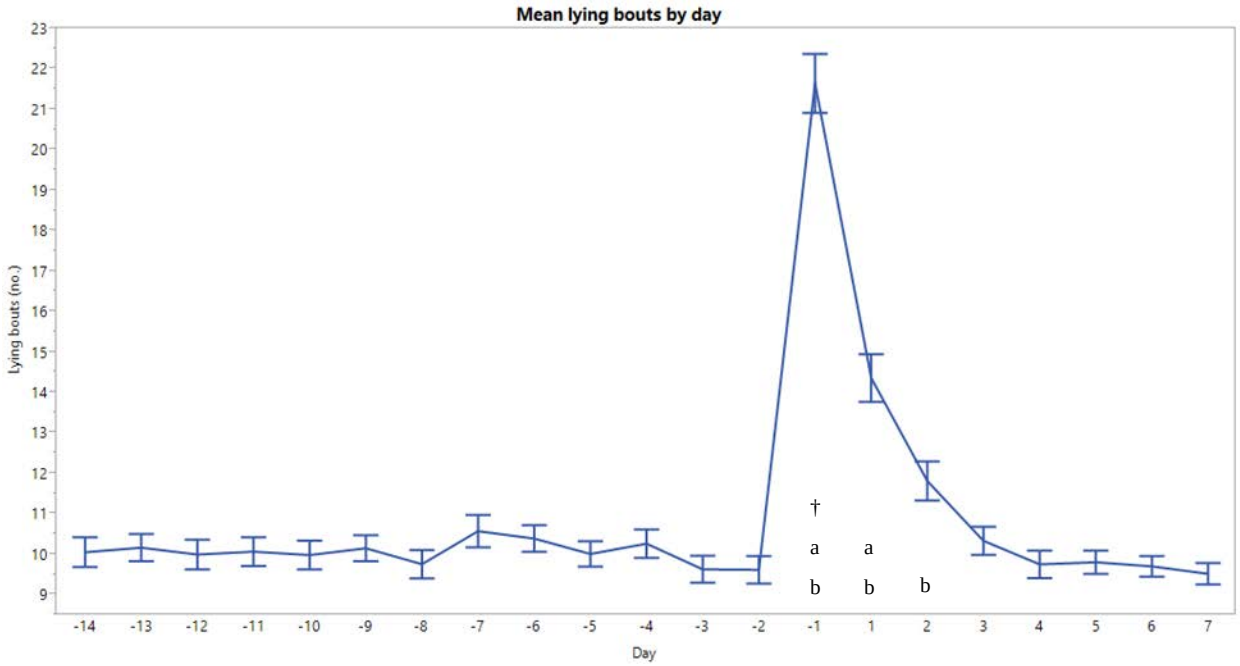


Figure 11. Mean lying bouts by day. Data from cows and heifers combined, n = 70. Superscript letters indicate: [a] Lying bouts on day -1 and 1 differed from days -14 to -2 ($P < 0.0001$); [b] Day -1, 1, and 2 differed from days 3 – 7. Superscript symbols indicate: [†] Median lying bout frequency between -24 and -16 hours differed from day -14 to -2 and day 3 – 7 ($P < 0.0001$).



Chapter 5: Manuscript: Characterization of neonatal beef calf behavior and associations with weight gain and intake of colostral immunoglobulins

Jenna E. Bayne, DVM, DACVIM[1], Paul H. Walz, DVM, PhD, DACVIM[1], Thomas Passler, DVM, PhD, DACVIM[1], Manuel F. Chamorro, DVM, MS, PhD, DACVIM[2], Deborah M. Haines, DVM, MPhil, PhD[3]

[1] College of Veterinary Medicine, Departments of Clinical Sciences and Pathobiology, Auburn University, Auburn, AL, USA

[2] College of Veterinary Medicine, Department of Clinical Sciences, Kansas State University, Manhattan, KS, USA

[3] College of Veterinary Medicine, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, SK, Canada

Supported by American Association of Bovine Practitioners and Auburn University Interdisciplinary Research and Equipment Grants Program.

This manuscript represents a portion of the dissertation submitted by Dr. Bayne to the Auburn University Graduate School as partial fulfillment of the requirements for Doctor of Philosophy degree.

Presented in part at the American Association of Bovine Practitioners Annual Conference, Charlotte, NC, September, 2016.

Introduction

Calving management on U.S. beef cow-calf operations is of utmost importance to producers and veterinarians alike, as adverse events during the periparturient period can impact cow and calf morbidity and mortality. The impact of dystocia and related conditions on cow and calf health are well-documented[573-576,600], with dystocia constituting the leading cause of death in beef calves.[574,575,577] Reported dystocia rates in beef cattle for which intervention was required range from less than 5% to over 18%.[573,575,576,578,579] Prolonged delivery with resultant hypoxia and respiratory acidosis has been associated with decreased IgG absorption.[601-603] Since calves are born agammaglobulinemic, timely ingestion of colostrum and absorption of immunoglobulins is critical for protection against infectious diseases. Failure to consume and absorb a sufficient volume of colostrum with adequate IgG concentration within the first several hours of life can lead to partial or complete failure of passive transfer of immunity (FPT) in calves.[604] Severe cold stress can also negatively affect calf vitality and the absorption of immunoglobulins.[605,606] Based on extensive research, FPT has been demonstrated to be an important risk factor for increased morbidity and mortality, predisposing pre-weaned calves to gastrointestinal and respiratory diseases. In addition, FPT in calves has been associated with reduced beef cow-calf productivity, including reduced average daily gains (ADG), decreased first and second lactation milk yields, and an increased rate of culling.[607-615]

Prevalence of FPT among beef calves in North America ranges from 11% to 31%[616] and is estimated to be 20 to 40% on U.S. dairy operations.[617,618] Concerted efforts focusing on farm-level management practices and producer education have translated into demonstrable improvement in diminishing risk of FPT on U.S. dairies.[617] Such efforts have emphasized established key factors that contribute to successful passive transfer of immunity: feeding

colostrum with a high immunoglobulin concentration (>50 g/L of IgG), feeding an adequate volume of colostrum, timely feeding of colostrum within the first several hours of life, and providing clean and quality colostrum through minimizing bacterial contamination.[604,619] Several assays have been used to determine the level of passive transfer of immunity in calves, with the use of radial immunodiffusion (RID) considered the gold standard.[604,620] A practical on-farm method is measurement of serum total protein (TP) by refractometer.[620] Adequate passive transfer of immunity is achieved at IgG concentrations of ≥ 10 g/L and TP of ≥ 5.2 g/dl, as measured at 1 to 7 days of age.[620] However, various cut-offs for adequate IgG and TP concentrations can be found in the literature as goals are continuously revised.[609,610]

Parturition in beef cattle on most U.S. cow-calf operations largely goes unsupervised, with visual appraisal of prepartum cows occurring only once to twice daily on average.[580] Thus, most neonatal calves are evaluated for the first time several hours after birth based on distant visual assessment and are directly handled even less often (e.g. for ear tattoo and tagging). Considering dam temperament, labor input, and pasture conditions, thorough examination of neonatal calves is frequently difficult for most producers. Practical and rapid assessment of calf vitality using various scoring systems, akin to Apgar scores used in human neonatology, has been described in calves, lambs, and piglets[621-629], but has not been extensively validated or standardized under different management conditions. Assessment may include: vital parameters (e.g. rectal temperature, heart and respiratory rates), muscle tone, stimulation and suckle reflexes, peripheral edema (e.g. swollen head, face), cyanosis of mucous membranes, and time to achieve sternal recumbency and standing.[626-630]

Objective evaluation of calf behavior as a measure of calf health using remote data capture technologies have been varied in their applications. Automated feeding systems, video

surveillance, and individual activity monitors have been utilized in the evaluation of ethophysiological, social-environmental interactions, and cognitive behaviors[367,631-635]; gait pattern assessment and activity monitoring[420-422]; and the impact of feeding regimes and housing conditions on heifer locomotor activities, feeding behavior, and performance.[369,370,636-644] Sickness behaviors have been evaluated under experimental conditions in post-weaned calves using these technologies following: endotoxin challenge[550]; *Mannheimia haemolytica*[412,462], *Mycoplasma bovis*[411], and bovine viral diarrhea virus (BVDV)[463] infection; as well as pain behavior associated with castration.[645,646] However, evaluation of pastured neonatal beef calf behavior using these technologies is absent. Application of remote activity monitors may have the potential to augment vitality assessment scores and quantification of immunoglobulin status for the identification of calves at risk of morbidity and mortality. The objective of the present study was to characterize the behavioral indices of neonatal beef calves during the first week of life using accelerometers. Evaluation of body weight, serum total protein and IgG concentrations were also performed to determine correlations between behavioral activity, status of passive immunity, and weight gain during the study period.

Materials and Methods

Experimental Design – All procedures were approved by the Auburn University Institutional Animal Care and Use Committee (2013-2366). The study was conducted at the Auburn University North Auburn Beef Unit (NABU) over two consecutive fall-calving seasons. Forty mixed-breed beef multiparous cows (first calving season, first year of study, mid-October to late November) and forty primiparous heifers (second calving season, second year of study, early September to mid-October) were enrolled in the study. Animals were maintained as one group, during respective seasons, on an 8-ha biosecure pasture throughout the study period. For

all animals, water and Bermuda grass hay were provided ad libitum, and a 12% protein concentration was fed once daily at a fixed time (08:00) to allow for distant visual assessment of each animal. The pasture was evaluated 3 times daily (approximately every 8 hours) for cows separated from the group and for the presence of newborn calves throughout the calving season. However, parturition was intended to be unobserved, as to minimize interference of human observation on calving behavior in beef cows. Therefore, the exact time of calving was undetermined. The date and time were recorded when the calf was found for each cow-calf pair throughout the study. Only calves born un-assisted were enrolled in the study. On day 1, calves were identified with an ear tag, weighed, bled, and an accelerometer was affixed to the right hind limb as soon as possible after birth. On day 7, calves were restrained, weighed, bled, and the accelerometer removed and data downloaded.

Body weight

Body weight was determined on days 1 and 7 using a manual hanging scale and sling--type harness to suspend the calf fully off the ground.

Blood sample collection

From each calf, a blood sample was collected by jugular venipuncture into an evacuated blood collection tube without additives (10 mL) on days 1 and 7. Blood samples were centrifuged, serum removed, and aliquots of serum samples obtained on days 1 and 7 were frozen and stored at -80°C for determination of total protein and immunoglobulin G concentrations by refractometry and single radial immunodiffusion (SID) assay, respectively, at the conclusion of the study period.

Single Radial Immunodiffusion

Serum (diluted 1:4) was assayed for total IgG concentration by single radial immunodiffusion, as previously described[647]. Antiserum against bovine IgG (H and L chain) was used (Jackson Laboratories Inc., West Grove, PA). Single radial immunodiffusion plates were prepared from 2% agarose containing 2.5% antiserum in phosphate buffered saline (PBS, pH=7.25). Standard curves (1.06-8.5 g/L) were produced using duplicate samples of a bovine IgG serum calibrator (Midlands Bio Products Corp., Boone, Iowa). The validity of plates was assessed with a reference serum from the Center for Veterinary Biologics, USDA APHIS. All samples were tested in triplicate and incubated in a humid atmosphere at 25ºC for 18-24 h. Ring diameters were measured with a computer assisted plate reader (The Binding Site, Birmingham, England) and the values in samples calculated using a program for linear analysis.

Accelerometers

A commercially manufactured accelerometer (IceQube, IceRobotics™) was attached to the lateral aspect of the right hind limb just proximal to the metacarpophalangeal joint, as previously described [414,422], for continuous activity monitoring throughout the study period. The device was placed in a light-weight mesh case and affixed to the limb using zip-ties. Accelerometers were removed on day 7 and data were downloaded to a computer. The data were continuously collected by the device and reported in 1-h intervals. Quantification of activity indices throughout the 7-day study period for each calf included time spent standing, time spent lying, number of steps, and number of times the calf transitioned from standing to lying positions (i.e., lying bouts) per 1-h time block.

Data analysis

Data were based on 1-h blocks, with each day of the 7-day observation period comprised of 24, 1-h blocks. The data were assessed for normality by visual inspection of frequency distribution and by the Shapiro-Wilk test as implemented in JMP 11.0.0 (SAS Institute, Cary, NC). Normally distributed variables included weight gain, serum IgG concentration, and sums of activity indices. The following variables were not normally distributed: standing time, lying time, walking time, steps, and lying bouts. For each day, the median time each calf spent lying, standing, and walking were calculated. Similarly, medians for lying bouts and steps taken were calculated. Comparison of each behavior by day over the 7-d observational period were made using the Friedman test as implemented in Prism 7 (GraphPad Software, San Diego, CA). Evaluation for the interaction of calf, dam parity group, and sums of behavioral activity indices were performed using the full-factorial (mixed model) function, as implemented in JMP 11.0.0 (SAS Institute, Cary, NC). Correlations between behavioral indices were determined using Spearman's rank correlation test as implemented in JMP 11.0.0. Pearson product moment correlations were determined for the parametric data of weight gain and serum IgG concentration. A Student's t-test was calculated for comparisons made between dam parity group and weight gain or serum IgG concentration, respectively. All data analyses were performed using commercial statistical software programs (JMP 11.0.0; Prism 7).

Results

Data were collected from 70 beef calves from two successive fall calving seasons. 37 calves (19 heifer calves, 18 bull calves) were born to multiparous cows in the first calving season and 33 calves (15 heifer calves, 18 bull calves) were born to primiparous heifers in the second calving season. In multiparous dams, three calves were not enrolled because of stillbirth (n = 2) or concealment of the calf by its dam for the first 2 days of life (n = 1). In primiparous dams,

calves not enrolled (n = 7) included: dystocia (n = 3), stillbirth (n = 3), and calf rejection by dam (n = 1). Placement, retention and use of accelerometers were tolerated well by all calves in the study. All behavioral data from the 70 calves enrolled were included in data analyses. Morbidity or mortality was not observed in any of the calves during the respective 7-day observation periods.

Physiological data

Serum IgG concentrations of calves born to multiparous cows was significantly greater than those of calves born to primiparous heifers, at 32 g/L compared to 19 g/L, respectively ($P < 0.0001$). Using the cut-off of 16g/L to represent adequate passive transfer of immunity and values <10 g/L inadequate, 34/37 (92%) calves from multiparous dams had adequate IgG concentrations on day 7. Of the 3 calves from multiparous dams with inadequate levels, 2 had marginal IgG concentrations (10 – 15 g/L) on day 1 but not on day 7 (<10 g/L). Only 20/33 (61%) calves born to primiparous dams had adequate IgG concentrations on day 7, with 7 and 6 calves having marginal and inadequate IgG concentrations on day 7, respectively. Calves born to multiparous cows had significantly greater weight gains compared to calves born to primiparous heifers during the first week of life (22.9lbs ± 1.47 vs. 18.4lbs ± 1.49, respectively, $P < 0.019$). A significant positive correlation existed between change in body weight and IgG status for both calves of multiparous cows and calves of primiparous heifers (Pearson's product correlation 0.5511, $P = 0.0006$ and 0.4090, $P = 0.0181$, respectively).

Behavioral data

Significant differences in calf behavioral activity indices were not observed between parity groups, thus data from all 70 calves were combined for descriptive statistical analyses

(Tables 3A-6A, 3B-6B). Overall, a gradual increase in the average time spent standing per day was observed during the study period (Table 3A; Figure 17). Similarly, increasing median time spent standing per hour was observed by day during the study period (Table 1B; Figure 12). Median time spent standing per hour was significantly different between multiple days. Days 1, 2, and 3 differed significantly from days 4 – 7, and day 4 differed from day 7 ($P < 0.05$, Figure 13). The mean standing time varied significantly by day (Figure 17). Day 1 differed significantly from days 5-7, and day 3 from days 6 and 7 ($P < 0.05$, Figure 17). Throughout the study period, calves spent the majority of the time lying down (Table 4A and 4B; Figure 12 and 18). Overall, a gradual decrease in the average time spent lying per day was observed (Figure 18). Significant differences in median lying time per hour were observed between multiple days. Median lying times on days 1, 2, and 3 differed significantly from days 4 – 7 ($P < 0.05$), and day 4 differed significantly from day 7 ($P < 0.05$; Figure 14). Significant differences in mean lying time by day were found (Figure 18). Day 1 differed significantly from days 6 and 7, day 2 from day 7, and day 3 from days 6 and 7 ($P < 0.05$; Figure 18). Average lying bout frequency was highest on day 1, followed by a dramatic drop in average lying bouts on days 2 – 7, with a gradual increase over time (Figure 20). Median lying bout frequency per hour was significantly different between day 1 and day 2, with a decrease in the lying bout frequency on day 2 compared to day 1 ($P < 0.05$, Figure 15). A significant difference in lying bout frequencies was present on day 2 compared to days 4 – 6, with a gradual increase in frequency during the latter part of the study period ($P < 0.05$, Figure 15). Median steps per hour differed significantly on day 1 compared to days 2, 3, and 7 ($P < 0.05$, Figure 16). Day 2 was different from day 4 – 7 ($P < 0.05$, Figure 16). Day 3 differed from day 1, 5 – 7 and day 4 and 5 differed from day 7, respectively ($P < 0.05$, Figure

16). Overall, average daily step counts gradually increased during the study period ($P < 0.02$, Figure 19).

Correlations between activity indices and IgG concentration, as well as activity indices and body weight were calculated. A significant positive correlation existed between lying time and IgG concentration for calves born to multiparous cows ($P = 0.5599$, $P = 0.0004$), but not for calves born to primiparous heifers ($P = -0.0443$, $P = 0.8066$). Similarly, standing time was negatively correlated with serum IgG concentration in calves born to multiparous cows ($P = -0.5599$, $P = 0.0004$), but not significantly different in calves born to primiparous heifers ($P = 0.0500$, $P = 0.7824$). Standing or lying times were not significantly correlated with weight gain during the first week of life. Although not statistically significant, a negative trend was observed for the number of steps taken and weight gain in calves born to multiparous cows ($P = -0.3030$, $P = 0.0769$). This trend was not observed in calves born to primiparous heifers ($P = 0.1506$, $P = 0.3953$).

Discussion

The major objective of the present study was to characterize the behavioral activity of neonatal beef calves during the first week of life using accelerometers. Behavioral activity was evaluated in conjunction with measurements of IgG concentrations and weight gains to evaluate correlations between behavioral indices, passive transfer of immunity, and overall calf health. This study is unique because it evaluated pastured beef calves, as activity monitors have predominately been utilized in adult cattle and much less so in preweaned calves. Placement, retention, and use of accelerometers were tolerated well by all calves in the study. Expectedly, behavioral activity data demonstrated that calves spent the vast majority of time lying during the first 7 days of life. Calves gradually spent more time standing, with a reciprocal decrease in time

spent lying. Although calves spent less time spent lying, lying bout frequency also gradually increased, with a resulting reduction in the amount of time spent lying per lying bout, as the study period proceeded. This is in agreement with the findings of Hill et al., which detected a gradual increase in the time spent standing with increasing age by approximately 0.5 min/day in individually housed, 2-5-day-old dairy calves as measured over a 60-day study period.[648] Similarly, Bonk et al. reported that 3-4-week-old, group-housed dairy calves spent 71 to 79% of the day lying down.[422]

Differences between parity groups with respect to correlations between behavioral activity indices and IgG status and weight gain were identified. Calves born to multiparous cows had significantly higher IgG concentrations and weight gains compared to calves born to primiparous heifers, which is supported[649] and contrasted[616] by previous literature. Only data from calves born without the need for assistance were included for analyses. Calvings were unattended and the possibility exists that data from prolonged but successful calvings, which could have negatively affected colostral intake or absorption by calves, were included. Presumably, this may have occurred more often in primiparous compared to multiparous dams. Colostral IgG concentrations was found to increase with parity in beef cows[650], which could also explain differences observed in calf serum IgG concentrations between parity groups. However, the effects of dam parity on colostral IgG concentration can be variable.[651-653] Dewell et al. detected a significant relationship between serum IgG1 concentration and preweaning weight gain, with a 3.4 kg increase in weight gain in calves with IgG1 concentrations >2,700 mg/dL.[609] While only speculative, the positive correlation found between lying time and IgG status in calves born to multiparous cows in this study might represent calves that experience adequate satiety and are therefore less likely to actively search for their dam. Similarly, a trend of fewer

steps was observed in calves of multiparous cows that gained greater weight over the first week of life. Similar results were not detected in calves born to primiparous heifers. The present findings are in contrast to Murray et al., who detected significantly lower weight gains in calves that spent less time standing during the first 2 days of life.[654] However, that study evaluated individually housed, bottle-fed dairy calves.[654] As well, it should be noted that previous research using similar activity monitors in calves detected the device to be less accurate for the quantification of walking and steps compared to the postural behaviors of lying and standing[420], and caution is warranted in interpreting the present trend in steps. Moreover, correlation does not imply causation, and IgG status and weight gain are impacted by multiple factors, including genetic potential, sex of calf, and milk production by the dam.

Unexpectedly, no significant differences between dam parity groups were detected in the median duration of behavioral activities of calves, such as time spent lying, standing, or steps. Hypothetically, calves could have been well-hidden and left relatively undisturbed more often by multiparous dams compared to calves more closely attended by primiparous dams, which could influence calf lying and standing behaviors. However, the opposite could be equally conceivable. The impact of maternal characteristics and mothering ability on calf activity indices is unknown. Given the adequate IgG concentrations found in calves from both parity groups, mothering ability was likely adequate in both primiparous and multiparous dams. Given the small sample size and relative uniformity of the herd, subtle differences in calf behavior due to maternal characteristics may have been difficult to detect.

Use of activity monitors and other remote technologies holds the potential to detect behavioral and physiological changes in neonatal beef calves predictive of sickness, complementing other means currently applied. Other tools include clinical assessment, such as

the use of calf vitality scores, for the identification of calves at risk for FPT and failure to thrive, allowing timely implementation of nursing and supportive care. As demonstrated by Schuijt et al. in attended calvings, time required to attain sternal recumbency in newborn calves is predictive of calf viability and practical in its application during the first 15 minutes of life.[626] Regarding calf vitality and morbidity, video surveillance of calvings under field conditions, Barrier et al. demonstrated reduced vigor in calves delivered with assistance compared to those delivered naturally as assessed by time to standing and suckling, as well as time spent lying in a lateral position.[655] However, detection of sickness behaviors predictive of morbidity in neonatal calves was not an objective of the present study, and morbidity was not observed in any of the calves. Further research is necessary to assess the capabilities of remote activity monitoring technologies to detect behavioral changes associated with morbidity in suckling beef calves under extensive pasture conditions.

The present study demonstrated neonatal beef calf behavior under pasture conditions using three-dimensional accelerometers. Calves spend the majority of the time lying down, with a gradual increase in the time spent standing during the first 7 days of life. The frequency of lying bouts increased, with a concurrent reduction in the time spent lying during each lying bout. Correlations between IgG concentration, weight gain, and activity indices were found in calves born to multiparous dams. No significant differences were found in the activity indices of calves born to primiparous dams compared to calves born to multiparous dams.

Activity Indices Tables

Table 3A. Mean standing time per study day based on 24-hr time periods, n = 70 calves.

Day	Mean	Standard Error
1	356.2	19.20
2	389.5	17.93
3	370.2	14.40
4	423.7	18.041
5	436.7	23.71
6	457.1	25.08
7	472.5	23.73

Table 3B. Median standing time per study day based on 1-hr time periods, n = 70 calves.

Day	Median	Interquartile Range	Standard Error
1	9.3	25.21	0.40
2	10.7	25.70	0.43
3	9.3	25.58	0.41
4	12.4	26.81	0.45
5	11.6	26.99	0.47
6	12.5	29.95	0.49
7	13.6	32.45	0.512

Table 4A. Mean lying time per study day based on 24-hr time periods, n = 70 calves.

Day	Mean	Standard Error
1	1083.8	19.20
2	1050.5	17.93
3	1069.8	14.40
4	1016.3	18.04
5	1003.3	23.71
6	981.1	25.50
7	943.3	26.21

Table 4B. Median calf lying time per study day based on 1-hr time periods, n = 70 calves.

Day	Median	Interquartile Range	Standard Error
1	50.7	25.21	0.40
2	49.3	25.70	0.43
3	50.7	25.58	0.41
4	47.6	26.81	0.45
5	48.4	26.99	0.47
6	47.5	29.95	0.49
7	46.3	32.47	0.51

Table 5A. Mean step count per study day based on 24-hr time periods, n = 70 calves.

Day	Mean	Standard Error
1	2601.1	86.47
2	2137.5	70.14
3	2279.3	70.12
4	2392.8	72.59
5	2488.4	82.91
6	2553.7	74.04
7	2765.4	105.07

Table 5B. Median step count per study day based on 1-hr time periods, n = 70 calves.

Day	Median	Interquartile Range	Standard Error
1	48	152	3.49
2	32	126	3.15
3	34	125	3.36
4	39	130	3.45
5	38	129.5	3.62
6	42	137	3.64
7	52	162	4.30

Table 6A. Mean lying bouts per study day based on 24-hr time periods, n = 70 calves.

Day	Mean	Standard Error
1	30.7	0.76
2	25.5	0.56
3	26.7	0.47
4	27.5	0.39
5	28.2	0.35
6	28.2	0.35
7	27.6	0.39

Table 6B. Median lying bouts per study day based on 1-hr time periods, n = 70 calves.

Day	Median	Interquartile Range	Standard Error
1	1	2	0.03
2	1	2	0.03
3	1	2	0.03
4	1	2	0.03
5	1	2	0.03
6	1	2	0.03
7	1	2	0.03

Figure 12. Median standing and lying time by study day based on 1-hr time periods, n = 70 calves.

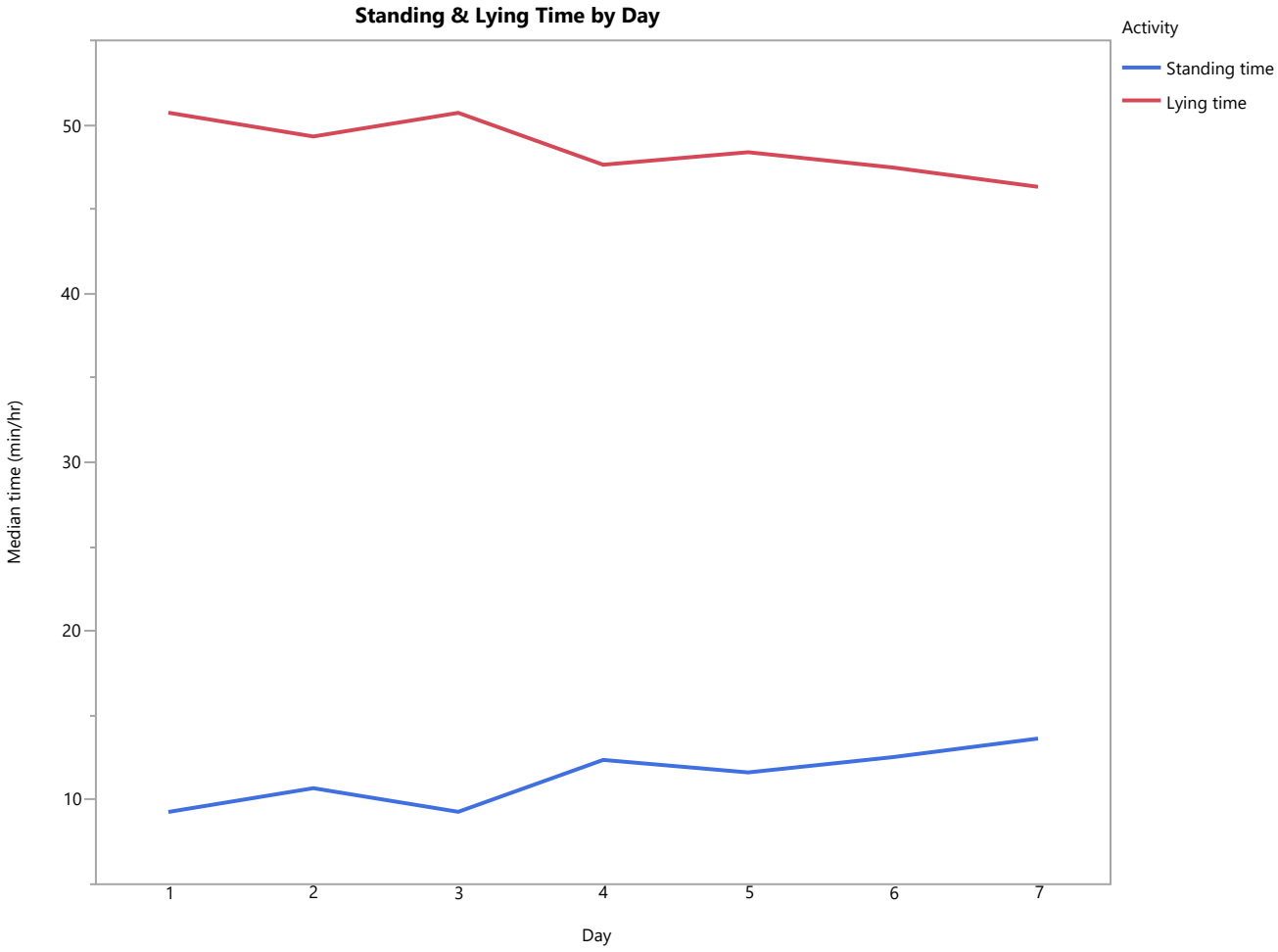


Figure 13. Median standing time per study day based on 1-hr time periods, n = 70 calves. Superscript letters indicate: [a] Days 1, 2, and 3 differed significantly from days 4 – 7 ($P < 0.05$).[b] Day 4 differed significantly from day 7 ($P < 0.05$).

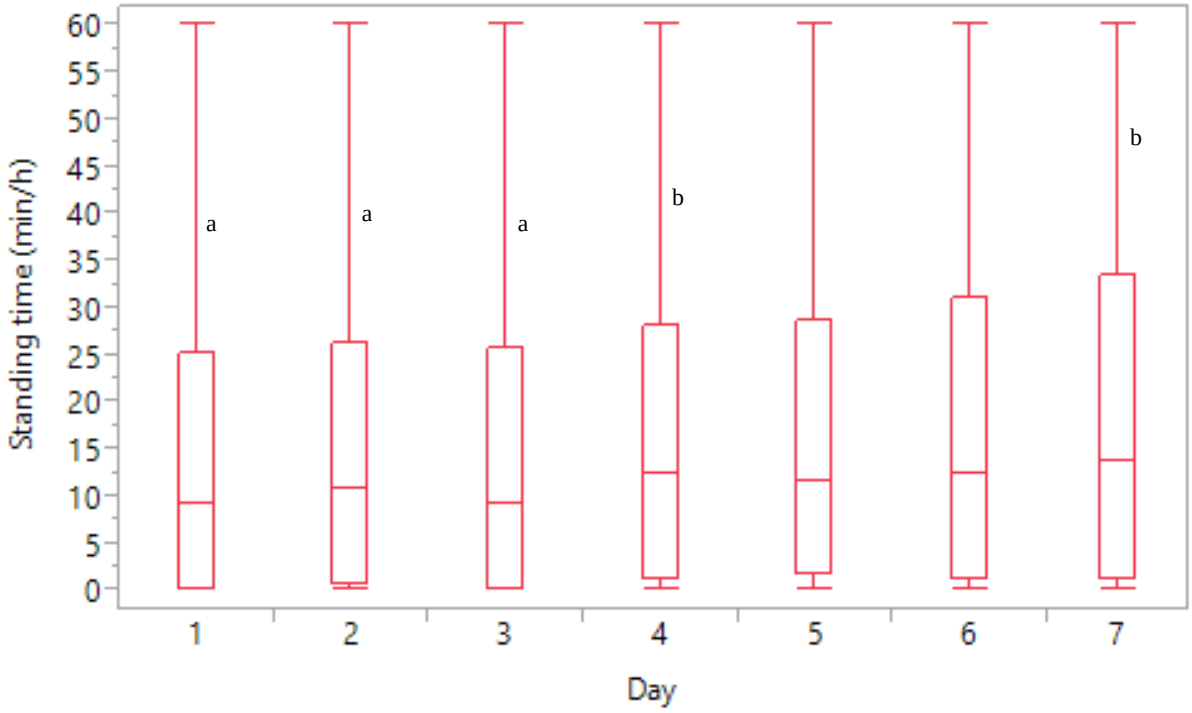


Figure 14. Median lying time per study day based on 1-hr time periods, n = 70 calves.

Superscript letters indicate: [a] Days 1, 2, and 3 differed significantly from days 4 – 7 ($P < 0.05$). [b] Day 4 differed significantly from day 7 ($P < 0.05$).

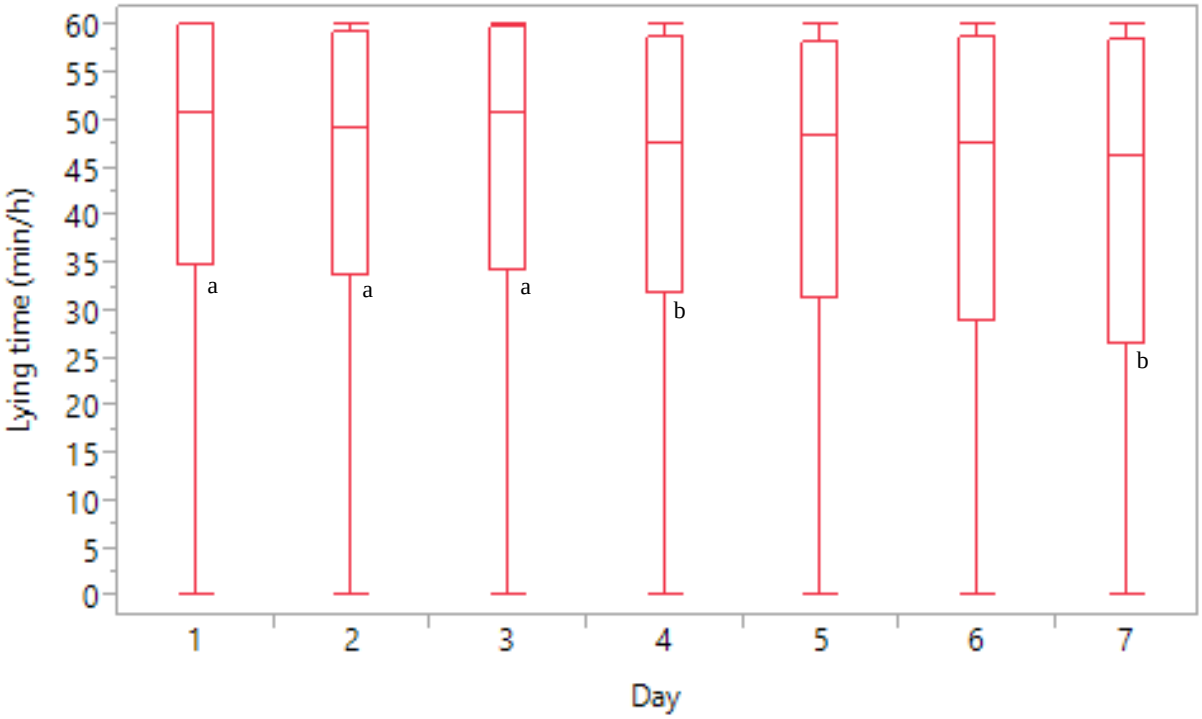


Figure 15. Median lying bouts per study day based on 1-hr time periods, n = 70 calves. Superscript letters indicate: [a] Day 1 significantly different from day 2 ($P < 0.05$). [b] Day 2 differed significantly from days 4 – 6 ($P < 0.05$).

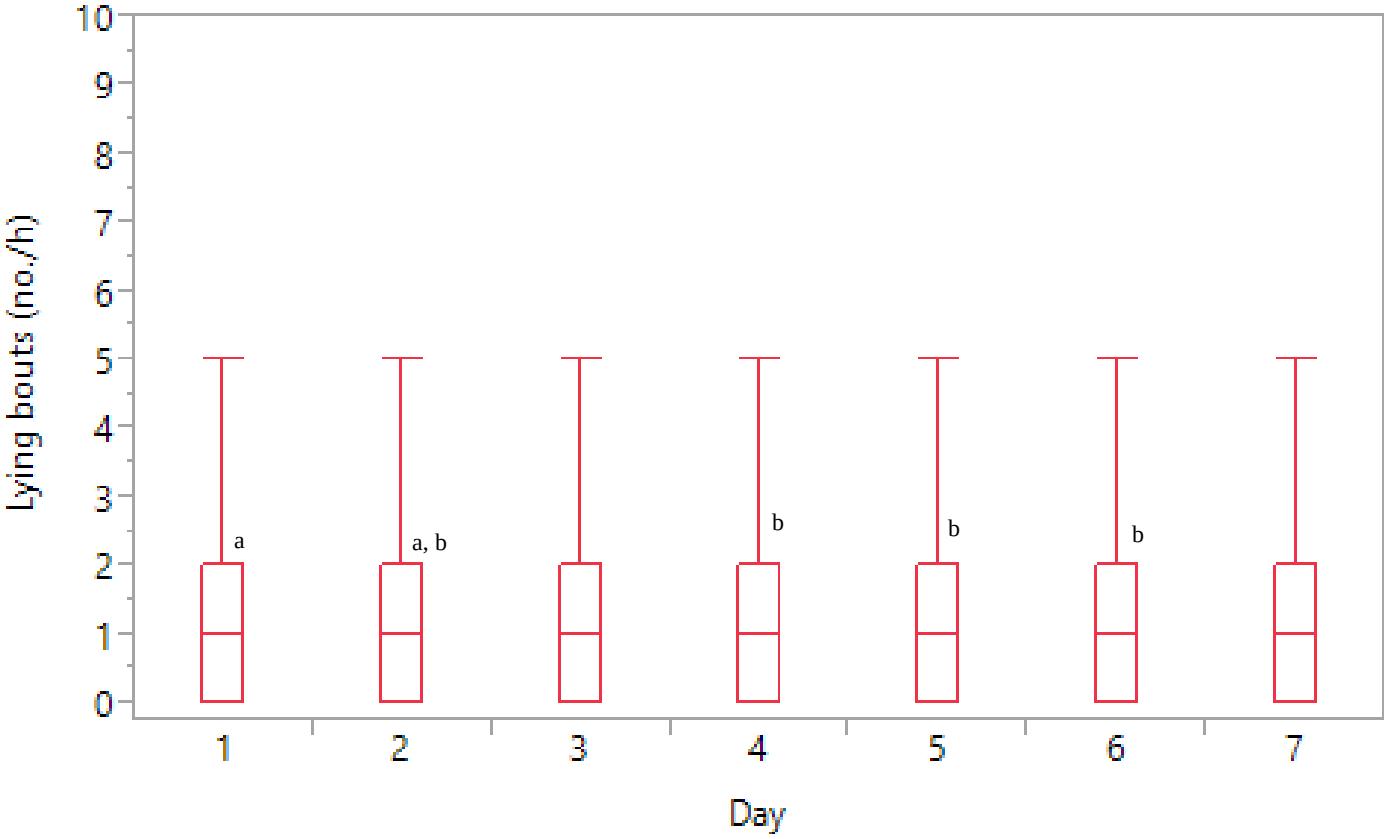


Figure 16. Median steps per study day based on 1-hr time periods, n = 70 calves. Superscript letters indicate: [a] Steps on day 1 were significantly different compared to days 2, 3, and 7 ($P < 0.05$). [b] Day 2 differed significantly from day 4 – 7 ($P < 0.05$). [c] Day 3 differed significantly from day 5 – 7 ($P < 0.05$). [d] Day 4 differed significantly from day 7 ($P < 0.05$). [e] Day 5 differed significantly from day 7 ($P < 0.05$).

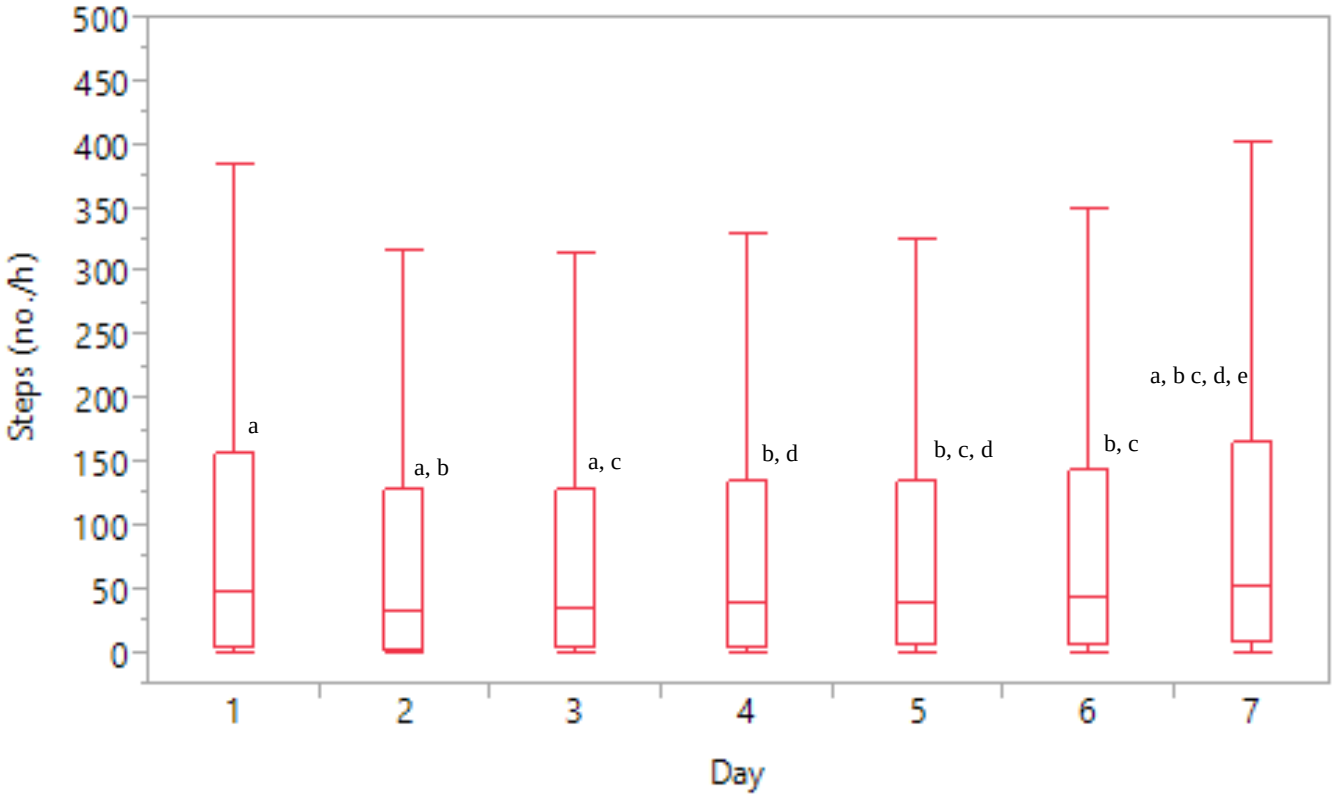


Figure 17. Mean standing time (+/- standard error) by day based on 24-hour time periods. N = 70 calves. Superscript letters indicate: [a] Day 1 differed significantly from days 5, 6, and 7 (*P* = 0.0494, 0.0058, and 0.0009, respectively). [b] Day 3 differed significantly from days 6 and 7 (*P* = 0.0289 and 0.0058, respectively).

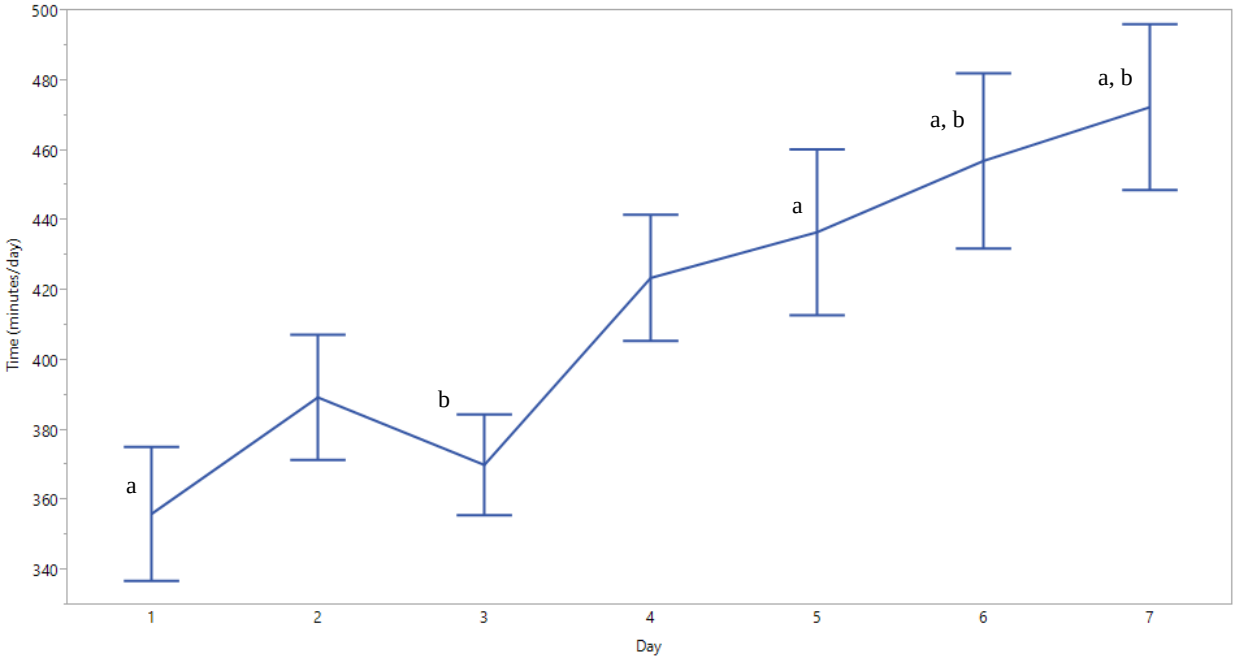


Figure 18. Mean lying time (+/- standard error) by day based on 24-hour time periods. N = 70 calves. Superscript letters indicate: [a] Day 1 differed significantly from days 6 and 7 (*P* = 0.0059 and < 0.0001, respectively). [b] Day 2 differed significantly from day 7 (*P* = 0.0042). [c] Day 3 differed significantly from days 6 and 7 (*P* = 0.0294 and 0.0003, respectively).

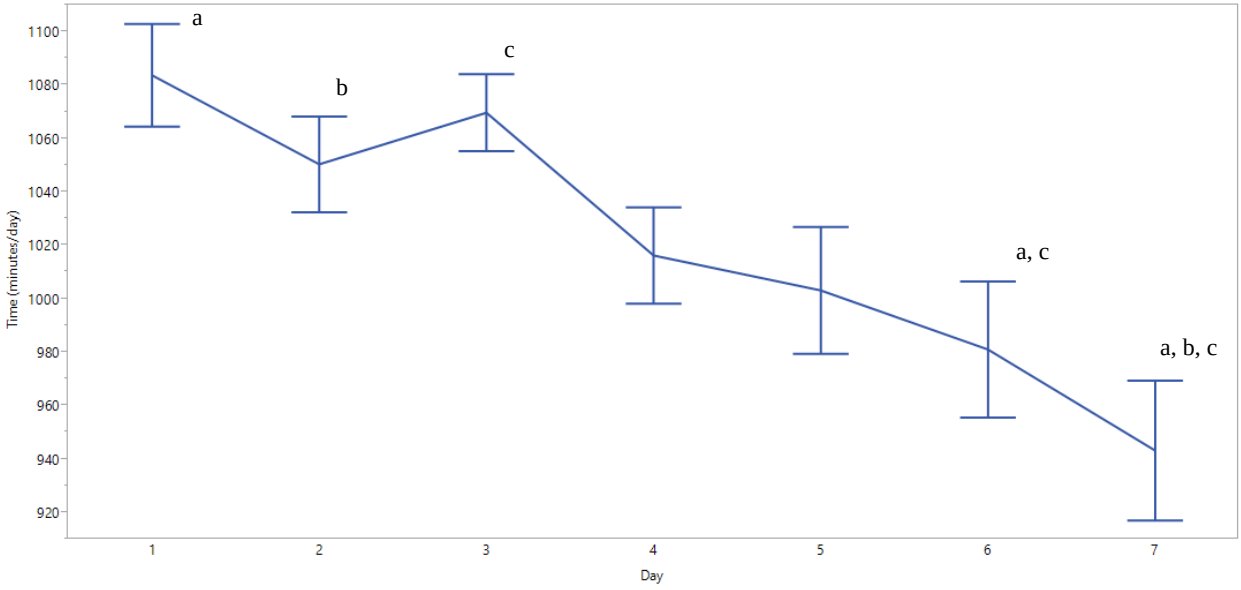


Figure 19. Mean steps (+/- standard error) by day based on 24-hour periods. N = 70 calves. Superscript letters indicate: [a] Day 1 differed significantly from days 2 and 3 ($P < 0.0001$ and 0.0134, respectively). [b] Day 2 differed significantly from days 5, 6, and 7 ($P = 0.0035$, 0.0003, and < 0.0001, respectively). [c] Day 3 differed significantly from day 7 ($P < 0.0001$). [d] Day 4 differed significantly from day 7 ($P = 0.0020$).

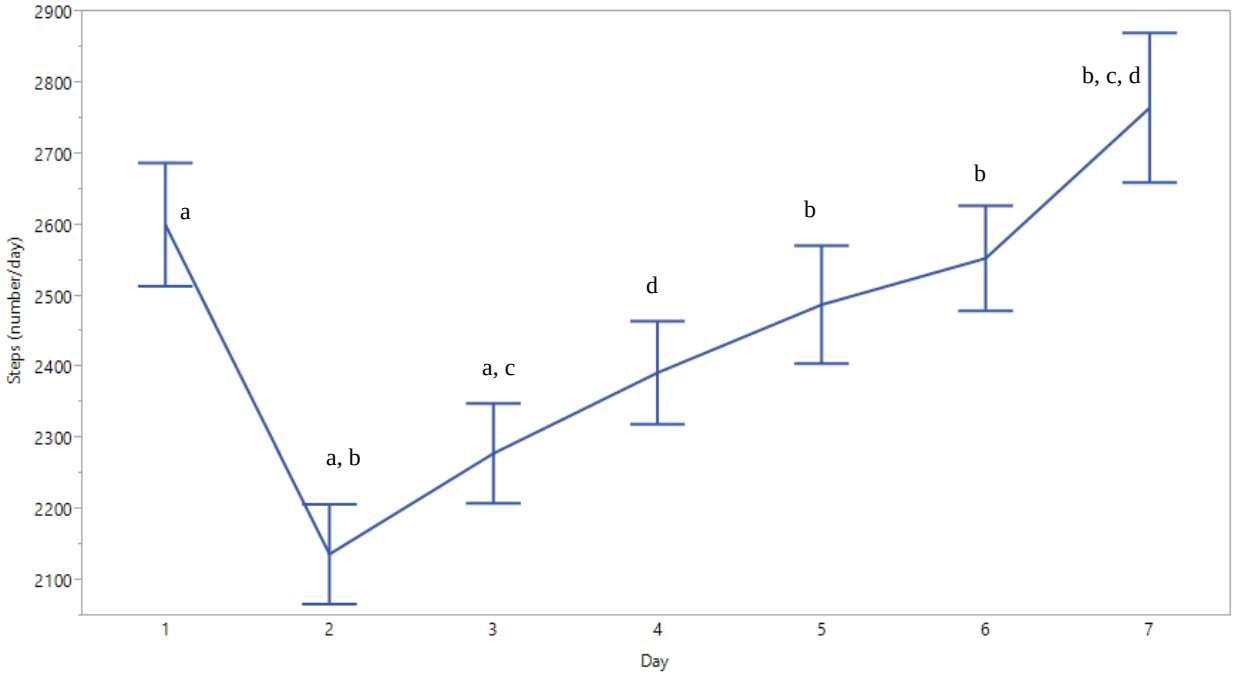
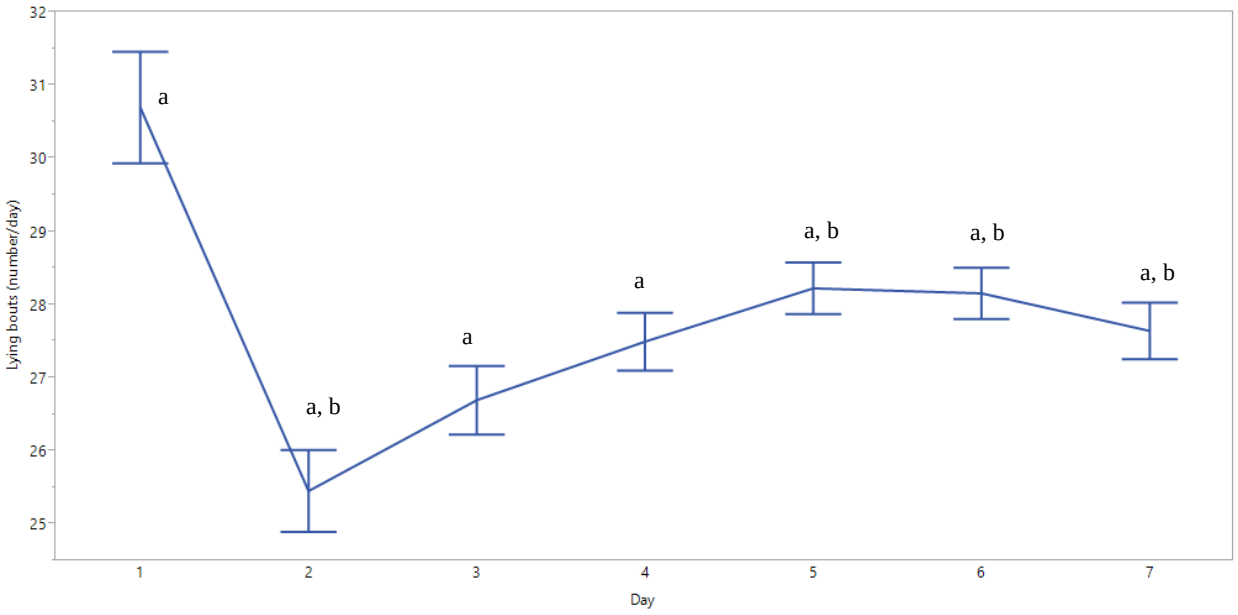


Figure 20. Mean lying bouts (+/- standard error) by day based on 24-hour periods. N = 70 calves. Superscript letters indicate: [a] Day 1 differed significantly from days 2 – 7 ($P < 0.0001$ day 2, 3, 4; $P = 0.0014$, day 5; $P = 0.0044$, day 6; $P = 0.0003$, day 7). [b] Day 2 differed significantly from day 5 – 7 ($P = 0.0014$, 0.0021, and 0.0341, respectively).



Chapter 6: Summary and Conclusions

Use of remote monitoring technologies is vastly expanding across the cattle industry, particularly in the dairy sector and the use of ever increasing precision farming tools. Significant contributors to the ongoing development and refinement of these technologies includes increasing operational size, with a diminishing skilled labor pool, along with progression in consumer interests over animal well-being and health. In review of the literature, technologies include temperature monitoring sensors, bunk attendance systems monitoring eating and drinking behaviors, rumination monitors, and devices used in the assessment of kinetic, kinematic, and overall behavioral activity of cattle. The latter includes accelerometers, which was the remote monitoring tool utilized in the studies presented herein. The overarching aim of the research of this dissertation was the characterization of beef cattle behavior using 3-dimensional accelerometers. Several classes of beef animals were evaluated: neonatal calves, weaned beef calves, and periparturient beef cows. Two experimental subclinical disease models were presented. First, the use of OMP facilitated low-dose, prolonged administration of endotoxin over 7 days to weaned beef calves. Second, weaned beef calves were intranasally inoculated with a low-virulence strain of BVDV. In both subclinical disease models, accelerometers were capable of demonstrating changes in behavior from baseline, following challenge. In conjunction with traditional markers of inflammation, accelerometers were a useful tool in the detection of very subtle behavioral changes which occurred during an APR of subclinical disease. In continuance, characterization of cattle behavior was carried out in periparturient beef cows and their calves over two consecutive calving seasons, also using

accelerometers. Similar to findings observed in housed dairy cows, pastured beef cattle demonstrated marked behavioral changes as parturition approached, based on activity data. The findings herein suggest that the use of accelerometers in pastured beef cattle could be useful during the periparturient period. Further research on the development of beef-specific algorithms for the prediction of parturition onset as well as the identification of dystocia-related behavioral changes is needed. Use of accelerometers in pregnant beef cattle could serve as another tool in maximizing the health and productivity of both cow and calf during the crucial periparturient period. Likewise, the use of accelerometers in beef calves demonstrated function in the descriptive analyses of behavior during the neonatal period. As well, associations were found between behavioral indices, colostrum intake, and weight gain in neonatal beef calves.

In conclusion, the studies presented within this dissertation add to the body of research evaluating the use of remote monitoring technologies in cattle. Specifically, the use of accelerometers for the characterization of sickness and normal physiological behavior was successfully demonstrated in pastured beef cattle. Future research is needed to expand the development of activity monitor-based behavioral data sets and algorithms applied to the analyses of these data for the prediction and identification of morbidity. As well, further research is needed in the application of these technologies under extensive management conditions often encountered on beef operations, with the aim of improving overall productivity, health, and well-being of beef cattle.

References

1 Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **124**, 783-801, doi:10.1016/j.cell.2006.02.015 (2006).

2 Linde, A. *et al.* Innate immunity and host defense peptides in veterinary medicine. *J. Vet. Intern. Med.* **22**, 247-265, doi:10.1111/j.1939-1676.2007.0038.x (2008).

3 Janeway, C. A., Jr. & Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **20**, 197-216, doi:10.1146/annurev.immunol.20.083001.084359 (2002).

4 Lien, E. & Ingalls, R. R. Toll-like receptors. *Crit. Care Med.* **30**, S1-11 (2002).

5 Matzinger, P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991-1045, doi:10.1146/annurev.iy.12.040194.005015 (1994).

6 Lewis, D. H., Chan, D. L., Pinheiro, D., Armitage-Chan, E. & Garden, O. A. The immunopathology of sepsis: pathogen recognition, systemic inflammation, the compensatory anti-inflammatory response, and regulatory T cells. *J. Vet. Intern. Med.* **26**, 457-482, doi:10.1111/j.1939-1676.2012.00905.x (2012).

7 Poltorak, A. *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* **282**, 2085-2088 (1998).

8 Takeda, K. & Akira, S. TLR signaling pathways. *Semin. Immunol.* **16**, 3-9 (2004).

9 Takeuchi, O. *et al.* Cellular responses to bacterial cell wall components are mediated through MyD88-dependent signaling cascades. *Int. Immunol.* **12**, 113-117 (2000).

10 Rietschel, E. T. *et al.* Bacterial endotoxin: molecular relationships of structure to activity and function. *FASEB J.* **8**, 217-225 (1994).

11 Donnelly, R. P., Fenton, M. J., Kaufman, J. D. & Gerrard, T. L. IL-1 expression in human monocytes is transcriptionally and posttranscriptionally regulated by IL-4. *J. Immunol.* **146**, 3431-3436 (1991).

12 Fenton, M. J., Buras, J. A. & Donnelly, R. P. IL-4 reciprocally regulates IL-1 and IL-1 receptor antagonist expression in human monocytes. *J. Immunol.* **149**, 1283-1288 (1992).

13 Fenton, M. J., Vermeulen, M. W., Clark, B. D., Webb, A. C. & Auron, P. E. Human pro-IL-1 beta gene expression in monocytic cells is regulated by two distinct pathways. *J. Immunol.* **140**, 2267-2273 (1988).

14 Schilling, D., Thomas, K., Nixdorff, K., Vogel, S. N. & Fenton, M. J. Toll-like receptor 4 and Toll-IL-1 receptor domain-containing adapter protein (TIRAP)/myeloid differentiation protein 88 adapter-like (Mal) contribute to maximal IL-6 expression in macrophages. *J. Immunol.* **169**, 5874-5880 (2002).

15 Ohlsson, K., Bjork, P., Bergenfeldt, M., Hageman, R. & Thompson, R. C. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* **348**, 550-552, doi:10.1038/348550a0 (1990).

16 Eckersall, P. D. & Bell, R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Vet. J.* **185**, 23-27, doi:10.1016/j.tvjl.2010.04.009 (2010).

17 Hart, B. L. Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* **12**, 123-137 (1988).

18 Dantzer, R. *et al.* Molecular basis of sickness behavior. *Ann. N. Y. Acad. Sci.* **856**, 132-138 (1998).

19 Pavlov, V. A. & Tracey, K. J. Neural circuitry and immunity. *Immunol. Res.* **63**, 38-57, doi:10.1007/s12026-015-8718-1 (2015).

20 Kent, S. *et al.* Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 9117-9120 (1992).

21 Besedovsky, H., del Rey, A., Sorkin, E. & Dinarello, C. A. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* **233**, 652-654 (1986).

22 Berkenbosch, F., van Oers, J., del Rey, A., Tilders, F. & Besedovsky, H. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science* **238**, 524-526 (1987).

23 Dantzer, R. & Kelley, K. W. Stress and immunity: an integrated view of relationships between the brain and the immune system. *Life Sci.* **44**, 1995-2008 (1989).

24 Dantzer, R. Cytokine, sickness behavior, and depression. *Immunol. Allergy Clin. North Am.* **29**, 247-264, doi:10.1016/j.iac.2009.02.002 (2009).

25 Bluthe, R. M., Michaud, B., Kelley, K. W. & Dantzer, R. Vagotomy attenuates behavioural effects of interleukin-1 injected peripherally but not centrally. *Neuroreport* **7**, 1485-1488 (1996).

26 Bluthe, R. M., Michaud, B., Kelley, K. W. & Dantzer, R. Vagotomy blocks behavioural effects of interleukin-1 injected via the intraperitoneal route but not via other systemic routes. *Neuroreport* **7**, 2823-2827 (1996).

27 Goehler, L. E., Gaykema, R. P., Hammack, S. E., Maier, S. F. & Watkins, L. R. Interleukin-1 induces c-Fos immunoreactivity in primary afferent neurons of the vagus nerve. *Brain Res.* **804**, 306-310 (1998).

28 Ek, M., Kurosawa, M., Lundeberg, T. & Ericsson, A. Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. *J. Neurosci.* **18**, 9471-9479 (1998).

29 Marvel, F. A., Chen, C. C., Badr, N., Gaykema, R. P. & Goehler, L. E. Reversible inactivation of the dorsal vagal complex blocks lipopolysaccharide-induced social withdrawal and c-Fos expression in central autonomic nuclei. *Brain. Behav. Immun.* **18**, 123-134, doi:10.1016/j.bbi.2003.09.004 (2004).

30 Romeo, H. E., Tio, D. L. & Taylor, A. N. Effects of glossopharyngeal nerve transection on central and peripheral cytokines and serum corticosterone induced by localized inflammation. *J. Neuroimmunol.* **136**, 104-111 (2003).

31 Konsman, J. P., Luheshi, G. N., Bluthe, R. M. & Dantzer, R. The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *Eur. J. Neurosci.* **12**, 4434-4446 (2000).

32 Konsman, J. P. *et al.* Central nervous action of interleukin-1 mediates activation of limbic structures and behavioural depression in response to peripheral administration of bacterial lipopolysaccharide. *Eur. J. Neurosci.* **28**, 2499-2510, doi:10.1111/j.1460-9568.2008.06549.x (2008).

33 Banks, W. A. The blood-brain barrier in psychoneuroimmunology. *Neurol. Clin.* **24**, 413-419, doi:10.1016/j.ncl.2006.03.009 (2006).

34 Romanovsky, A. A. Thermoregulatory manifestations of systemic inflammation: lessons from vagotomy. *Auton. Neurosci.* **85**, 39-48, doi:10.1016/s1566-0702(00)00218-6 (2000).

35 Tansey, E. A. & Johnson, C. D. Recent advances in thermoregulation. *Adv. Physiol. Educ.* **39**, 139-148, doi:10.1152/advan.00126.2014 (2015).

36 Elmquist, J. K., Scammell, T. E. & Saper, C. B. Mechanisms of CNS response to systemic immune challenge: the febrile response. *Trends Neurosci.* **20**, 565-570 (1997).

37 Dantzer, R. Cytokine, sickness behavior, and depression. *Neurol. Clin.* **24**, 441-460, doi:10.1016/j.ncl.2006.03.003 (2006).

38 Herman, J. P. & Seroogy, K. Hypothalamic-pituitary-adrenal axis, glucocorticoids, and neurologic disease. *Neurol. Clin.* **24**, 461-481, vi, doi:10.1016/j.ncl.2006.03.006 (2006).

39 Roth, J., Rummel, C., Barth, S. W., Gerstberger, R. & Hubschle, T. Molecular aspects of fever and hyperthermia. *Neurol. Clin.* **24**, 421-439, v, doi:10.1016/j.ncl.2006.03.004 (2006).

40 van den Top, M. & Spanswick, D. Integration of metabolic stimuli in the hypothalamic arcuate nucleus. *Prog. Brain Res.* **153**, 141-154, doi:10.1016/s0079-6123(06)53008-0 (2006).

41 Konsman, J. P., Kelley, K. & Dantzer, R. Temporal and spatial relationships between lipopolysaccharide-induced expression of Fos, interleukin-1beta and inducible nitric oxide synthase in rat brain. *Neuroscience* **89**, 535-548 (1999).

42 Laye, S. *et al.* Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **279**, R93-98 (2000).

43 Anforth, H. R. *et al.* Biological activity and brain actions of recombinant rat interleukin-1alpha and interleukin-1beta. *Eur. Cytokine Netw.* **9**, 279-288 (1998).

44 Burgess, W. *et al.* Interleukin-1beta-converting enzyme-deficient mice resist central but not systemic endotoxin-induced anorexia. *Am. J. Physiol.* **274**, R1829-1833 (1998).

45 Kim, Y. W. *et al.* Time-course changes of hormones and cytokines by lipopolysaccharide and its relation with anorexia. *J. Physiol. Sci.* **57**, 159-165, doi:10.2170/physiolsci.RP003407 (2007).

46 Swiergiel, A. H. & Dunn, A. J. Distinct roles for cyclooxygenases 1 and 2 in interleukin-1-induced behavioral changes. *J. Pharmacol. Exp. Ther.* **302**, 1031-1036, doi:10.1124/jpet.102.036640 (2002).

47 Dunn, A. J. & Swiergiel, A. H. The role of cyclooxygenases in endotoxin- and interleukin-1-induced hypophagia. *Brain. Behav. Immun.* **14**, 141-152, doi:10.1006/brbi.1999.0580 (2000).

48 Bazar, K. A., Yun, A. J. & Lee, P. Y. "Starve a fever and feed a cold": feeding and anorexia may be adaptive behavioral modulators of autonomic and T helper balance. *Med. Hypotheses* **64**, 1080-1084, doi:10.1016/j.mehy.2004.05.020 (2005).

49 Kahl, K. G., Kruse, N., Rieckmann, P. & Schmidt, M. H. Cytokine mRNA expression patterns in the disease course of female adolescents with anorexia nervosa. *Psychoneuroendocrinology* **29**, 13-20 (2004).

50 Tizard, I. Sickness behavior, its mechanisms and significance. *Anim. Health Res. Rev.* **9**, 87-99, doi:10.1017/s1466252308001448 (2008).

51 Freeman, L. M. Cachexia and sarcopenia: emerging syndromes of importance in dogs and cats. *J. Vet. Intern. Med.* **26**, 3-17, doi:10.1111/j.1939-1676.2011.00838.x (2012).

52 Melstrom, L. G., Melstrom, K. A., Jr., Ding, X. Z. & Adrian, T. E. Mechanisms of skeletal muscle degradation and its therapy in cancer cachexia. *Histol. Histopathol.* **22**, 805-814, doi:10.14670/hh-22.805 (2007).

53 Hamid, T. *et al.* Divergent tumor necrosis factor receptor-related remodeling responses in heart failure: role of nuclear factor-kappaB and inflammatory activation. *Circulation* **119**, 1386-1397, doi:10.1161/circulationaha.108.802918 (2009).

54 Moresi, V. *et al.* Tumor necrosis factor-alpha inhibition of skeletal muscle regeneration is mediated by a caspase-dependent stem cell response. *Stem Cells* **26**, 997-1008, doi:10.1634/stemcells.2007-0493 (2008).

55 Tisdale, M. J. Mechanisms of cancer cachexia. *Physiol. Rev.* **89**, 381-410, doi:10.1152/physrev.00016.2008 (2009).

56 Zhang, G. *et al.* Toll-like receptor 4 mediates Lewis lung carcinoma-induced muscle wasting via coordinate activation of protein degradation pathways. *Sci. Rep.* **7**, 2273, doi:10.1038/s41598-017-02347-2 (2017).

57 Popa, C., Netea, M. G., van Riel, P. L., van der Meer, J. W. & Stalenhoef, A. F. The role of TNF-alpha in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. *J. Lipid Res.* **48**, 751-762, doi:10.1194/jlr.R600021-JLR200 (2007).

58 Conde, J. *et al.* At the crossroad between immunity and metabolism: focus on leptin. *Expert Rev. Clin. Immunol.* **6**, 801-808, doi:10.1586/eci.10.48 (2010).

59 Perez-Perez, A. *et al.* Role of leptin as a link between metabolism and the immune system. *Cytokine Growth Factor Rev.* **35**, 71-84, doi:10.1016/j.cytogfr.2017.03.001 (2017).

60 Procaccini, C. *et al.* Leptin as immune mediator: Interaction between neuroendocrine and immune system. *Dev. Comp. Immunol.* **66**, 120-129, doi:http://dx.doi.org/10.1016/j.dci.2016.06.006 (2017).

61 Cremona, S., Laye, S., Dantzer, R. & Parnet, P. Blockade of brain type II interleukin-1 receptors potentiates IL1beta-induced anorexia in mice. *Neurosci. Lett.* **246**, 101-104 (1998).

62 Bluthe, R. M. *et al.* Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. *Eur. J. Neurosci.* **12**, 4447-4456 (2000).

63 Kubota, T., Kushikata, T., Fang, J. & Krueger, J. M. Nuclear factor-kappaB inhibitor peptide inhibits spontaneous and interleukin-1beta-induced sleep. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **279**, R404-413 (2000).

64 Nadjar, A., Bluthe, R. M., May, M. J., Dantzer, R. & Parnet, P. Inactivation of the cerebral NFkappaB pathway inhibits interleukin-1beta-induced sickness behavior and c-Fos expression in various brain nuclei. *Neuropsychopharmacology* **30**, 1492-1499, doi:10.1038/sj.npp.1300755 (2005).

65 Apley, M. Bovine respiratory disease: pathogenesis, clinical signs, and treatment in lightweight calves. *Vet. Clin. North Am. Food Anim. Pract.* **22**, 399-411, doi:10.1016/j.cvfa.2006.03.009 (2006).

66 Engler, M., Defoor, P., King, C. & Gleghorn, J. The impact of bovine respiratory disease: the current feedlot experience. *Anim. Health Res. Rev.* **15**, 126-129, doi:10.1017/s1466252314000139 (2014).

67 Timsit, E., Dendukuri, N., Schiller, I. & Buczinski, S. Diagnostic accuracy of clinical illness for bovine respiratory disease (BRD) diagnosis in beef cattle placed in feedlots: A systematic literature review and hierarchical Bayesian latent-class meta-analysis. *Prev. Vet. Med.* **135**, 67-73, doi:10.1016/j.prevetmed.2016.11.006 (2016).

68 Hayes, G., Mathews, K., Kruth, S., Doig, G. & Dewey, C. Illness severity scores in veterinary medicine: what can we learn? *J. Vet. Intern. Med.* **24**, 457-466, doi:10.1111/j.1939-1676.2010.0483.x (2010).

69 Amrine, D. E. *et al.* Precision and accuracy of clinical illness scores, compared with pulmonary consolidation scores, in Holstein calves with experimentally induced

Mycoplasma bovis pneumonia. *Am. J. Vet. Res.* **74**, 310-315, doi:10.2460/ajvr.74.2.310 (2013).

70 Buhman, M. J. *et al.* Association between changes in eating and drinking behaviors and respiratory tract disease in newly arrived calves at a feedlot. *Am. J. Vet. Res.* **61**, 1163-1168 (2000).

71 Step, D. L. *et al.* Effects of commingling beef calves from different sources and weaning protocols during a forty-two-day receiving period on performance and bovine respiratory disease. *J. Anim. Sci.* **86**, 3146-3158, doi:10.2527/jas.2008-0883 (2008).

72 Allen, J. W. *et al.* The microbial flora of the respiratory tract in feedlot calves: associations between nasopharyngeal and bronchoalveolar lavage cultures. *Can. J. Vet. Res.* **55**, 341-346 (1991).

73 Buczinski, S., Forte, G., Francoz, D. & Belanger, A. M. Comparison of thoracic auscultation, clinical score, and ultrasonography as indicators of bovine respiratory disease in preweaned dairy calves. *J. Vet. Intern. Med.* **28**, 234-242, doi:10.1111/jvim.12251 (2014).

74 Wittum, T. E., Woollen, N. E., Perino, L. J. & Littledike, E. T. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and rate of weight gain in feedlot cattle. *J. Am. Vet. Med. Assoc.* **209**, 814-818 (1996).

75 Schneider, M. J., Tait, R. G., Jr., Busby, W. D. & Reecy, J. M. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment records and lung lesion scores. *J. Anim. Sci.* **87**, 1821-1827, doi:10.2527/jas.2008-1283 (2009).

76 Taylor, J. D., Fulton, R. W., Lehenbauer, T. W., Step, D. L. & Confer, A. W. The epidemiology of bovine respiratory disease: What is the evidence for preventive measures? *The Canadian Veterinary Journal* **51**, 1351-1359 (2010).

77 Thompson, P. N., Stone, A. & Schultheiss, W. A. Use of treatment records and lung lesion scoring to estimate the effect of respiratory disease on growth during early and late finishing periods in South African feedlot cattle1. *J. Anim. Sci.* **84**, 488-498, doi:10.2527/2006.842488x (2006).

78 White, B. J. & Renter, D. G. Bayesian estimation of the performance of using clinical observations and harvest lung lesions for diagnosing bovine respiratory disease in post-

weaned beef calves. *J. Vet. Diagn. Invest.* **21**, 446-453, doi:10.1177/104063870902100405 (2009).

79 Gardner, B. A., Dolezal, H. G., Bryant, L. K., Owens, F. N. & Smith, R. A. Health of finishing steers: effects on performance, carcass traits, and meat tenderness. *J. Anim. Sci.* **77**, 3168-3175 (1999).

80 Bryant, L. K., Perino, L. J., Griffin, D., Doster, A. R. & Wittum, T. E. A method for recording pulmonary lesions of beef calves at slaughter, and the association of lesions with average daily gain *Bovine Practitioner* **33**, 163-173 (1999).

81 Buczinski, S., T, L. O. & Dendukuri, N. Bayesian estimation of the accuracy of the calf respiratory scoring chart and ultrasonography for the diagnosis of bovine respiratory disease in pre-weaned dairy calves. *Prev. Vet. Med.* **119**, 227-231, doi:10.1016/j.prevetmed.2015.02.018 (2015).

82 White, B. J. *et al.* Bayesian evaluation of clinical diagnostic test characteristics of visual observations and remote monitoring to diagnose bovine respiratory disease in beef calves. *Prev. Vet. Med.* **126**, 74-80, doi:http://dx.doi.org/10.1016/j.prevetmed.2016.01.027 (2016).

83 Theurer, M. E., Amrine, D. E. & White, B. J. Remote noninvasive assessment of pain and health status in cattle. *Vet. Clin. North Am. Food Anim. Pract.* **29**, 59-74, doi:10.1016/j.cvfa.2012.11.011 (2013).

84 Buczinski, S. *et al.* Assessment of L-lactatemia as a predictor of respiratory disease recognition and severity in feedlot steers. *Prev. Vet. Med.* **118**, 306-318, doi:10.1016/j.prevetmed.2014.12.003 (2015).

85 Aich, P., Babiuk, L. A., Potter, A. A. & Griebel, P. Biomarkers for prediction of bovine respiratory disease outcome. *OMICS* **13**, 199-209, doi:10.1089/omi.2009.0012 (2009).

86 Mang, A. V., Buczinski, S., Booker, C. W. & Timsit, E. Evaluation of a Computer-aided Lung Auscultation System for Diagnosis of Bovine Respiratory Disease in Feedlot Cattle. *J. Vet. Intern. Med.* **29**, 1112-1116, doi:10.1111/jvim.12657 (2015).

87 Wolfger, B. *et al.* Feeding behavior as an early predictor of bovine respiratory disease in North American feedlot systems. *J. Anim. Sci.* **93**, 377-385, doi:10.2527/jas.2013-8030 (2015).

88 Wolfger, B., Timsit, E., White, B. J. & Orsel, K. A Systematic Review of Bovine Respiratory Disease Diagnosis Focused on Diagnostic Confirmation, Early Detection, and Prediction of Unfavorable Outcomes in Feedlot Cattle. *Vet. Clin. North Am. Food Anim. Pract.* **31**, 351-365, v-vi, doi:10.1016/j.cvfa.2015.05.005 (2015).

89 Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340**, 448-454, doi:10.1056/nejm199902113400607 (1999).

90 Baumann, H., Prowse, K. R., Won, K. A., Marinkovic, S. & Jahreis, G. P. Regulation of acute phase protein genes by hepatocyte-stimulating factors, monokines and glucocorticoids. *Tokai J. Exp. Clin. Med.* **13**, 277-292 (1988).

91 Baumann, H., Richards, C. & Gauldie, J. Interaction among hepatocyte-stimulating factors, interleukin 1, and glucocorticoids for regulation of acute phase plasma proteins in human hepatoma (HepG2) cells. *J. Immunol.* **139**, 4122-4128 (1987).

92 Castell, J. V. *et al.* Recombinant human interleukin-6 (IL-6/BSF-2/HSF) regulates the synthesis of acute phase proteins in human hepatocytes. *FEBS Lett.* **232**, 347-350 (1988).

93 Morley, J. J. & Kushner, I. Serum C-reactive protein levels in disease. *Ann. N. Y. Acad. Sci.* **389**, 406-418 (1982).

94 Shamay, A. *et al.* Expression of albumin in nonhepatic tissues and its synthesis by the bovine mammary gland. *J. Dairy Sci.* **88**, 569-576, doi:10.3168/jds.S0022-0302(05)72719-3 (2005).

95 Csilla, T., Oskar, N., H, S., P, I. & Kovac, G. *The influence of hoof diseases on the concentrations of some acute phase proteins and other variables of the protein profile in heifers*. Vol. 61 (2011).

96 Arthington, J. D. *et al.* Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. *J. Anim. Sci.* **86**, 2016-2023, doi:10.2527/jas.2008-0968 (2008).

97 Coetzee, G. A. *et al.* Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. *J. Biol. Chem.* **261**, 9644-9651 (1986).

98 Dziegielewska, K. M. *et al.* Fetuin: an acute phase protein in cattle. *J. Comp. Physiol. B* **162**, 168-171 (1992).

99 Liang, J. S. & Sipe, J. D. Recombinant human serum amyloid A (apoSAAp) binds cholesterol and modulates cholesterol flux. *J. Lipid Res.* **36**, 37-46 (1995).

100 Manley, P. N., Ancsin, J. B. & Kisilevsky, R. Rapid recycling of cholesterol: the joint biologic role of C-reactive protein and serum amyloid A. *Med. Hypotheses* **66**, 784-792, doi:10.1016/j.mehy.2005.10.018 (2006).

101 Pineiro, M. *et al.* ITIH4 (inter-alpha-trypsin inhibitor heavy chain 4) is a new acute-phase protein isolated from cattle during experimental infection. *Infect. Immun.* **72**, 3777-3782, doi:10.1128/iai.72.7.3777-3782.2004 (2004).

102 Trevisi, E., Amadori, M., Bakudila, A. M. & Bertoni, G. Metabolic changes in dairy cows induced by oral, low-dose interferon-alpha treatment. *J. Anim. Sci.* **87**, 3020-3029, doi:10.2527/jas.2008-1178 (2009).

103 Conner, J. G., Eckersall, P. D., Wiseman, A., Bain, R. K. & Douglas, T. A. Acute phase response in calves following infection with Pasteurella haemolytica, Ostertagia ostertagi and endotoxin administration. *Res. Vet. Sci.* **47**, 203-207 (1989).

104 Orro, T. *et al.* Temporal changes in serum concentrations of acute phase proteins in newborn dairy calves. *Vet. J.* **176**, 182-187, doi:10.1016/j.tvjl.2007.02.010 (2008).

105 Itoh, H., Tamura, K., Izumi, M., Motoi, Y. & Funayama, Y. Characterization of serum alpha 1-acid glycoprotein in fetal and newborn calves during development. *Am. J. Vet. Res.* **54**, 591-595 (1993).

106 Alsemgeest, S. P. *et al.* Serum amyloid-A (SAA) and haptoglobin (Hp) plasma concentrations in newborn calves. *Theriogenology* **43**, 381-387 (1995).

107 Debski, B. *et al.* Evaluation of Acute Phase Proteins in Clinically Healthy Dairy Cows in Perinatal Period and During Lactation. *Pol. J. Vet. Sci.* **19**, 519-523, doi:10.1515/pjvs-2016-0065 (2016).

108 Saremi, B. *et al.* Bovine haptoglobin as an adipokine: serum concentrations and tissue expression in dairy cows receiving a conjugated linoleic acids supplement throughout lactation. *Vet. Immunol. Immunopathol.* **146**, 201-211, doi:10.1016/j.vetimm.2012.03.011 (2012).

109 Seppa-Lassila, L., Orro, T., Lepage, J. P. & Soveri, T. Reference values for acute phase proteins in calves and its clinical application. *Vet. Rec.* **173**, 319, doi:10.1136/vr.101233 (2013).

110 Thomas, F. C., Waterston, M., Hastie, P., Haining, H. & Eckersall, P. D. Early post parturient changes in milk acute phase proteins. *J. Dairy Res.* **83**, 352-359, doi:10.1017/s0022029916000297 (2016).

111 Murray, C. F. *et al.* Associations of serum haptoglobin in newborn dairy calves with health, growth, and mortality up to 4 months of age. *J. Dairy Sci.* **97**, 7844-7855, doi:10.3168/jds.2014-8465 (2014).

112 Hussein, H. A. & Staufenbiel, R. Variations in copper concentration and ceruloplasmin activity of dairy cows in relation to lactation stages with regard to ceruloplasmin to copper ratios. *Biol. Trace Elem. Res.* **146**, 47-52, doi:10.1007/s12011-011-9226-3 (2012).

113 McCarthy, M. M., Yasui, T., Felippe, M. J. & Overton, T. R. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. *J. Dairy Sci.* **99**, 680-700, doi:10.3168/jds.2015-9694 (2016).

114 Morimatsu, M. *et al.* Isolation and characterization of bovine haptoglobin from acute phase sera. *J. Biol. Chem.* **266**, 11833-11837 (1991).

115 Eckersall, P. D. & Conner, J. G. Plasma haptoglobin in cattle (Bos taurus) exists as polymers in association with albumin. *Comp. Biochem. Physiol. B* **96**, 309-314 (1990).

116 Huzzey, J. M. *et al.* Short communication: Haptoglobin as an early indicator of metritis. *J. Dairy Sci.* **92**, 621-625, doi:10.3168/jds.2008-1526 (2009).

117 Nakagawa-Tosa, N. *et al.* Stimulation of haptoglobin synthesis by interleukin-6 and tumor necrosis factor, but not by interleukin-1, in bovine primary cultured hepatocytes. *J. Vet. Med. Sci.* **57**, 219-223 (1995).

118 Lecchi, C., Dilda, F., Sartorelli, P. & Ceciliani, F. Widespread expression of SAA and Hp RNA in bovine tissues after evaluation of suitable reference genes. *Vet. Immunol. Immunopathol.* **145**, 556-562, doi:10.1016/j.vetimm.2011.12.017 (2012).

119 Dilda, F. *et al.* Distribution of acute phase proteins in the bovine forestomachs and abomasum. *Vet. J.* **192**, 101-105, doi:10.1016/j.tvjl.2011.05.005 (2012).

120 Lai, I. H. *et al.* Neutrophils as one of the major haptoglobin sources in mastitis affected milk. *Vet. Res.* **40**, 17, doi:10.1051/vetres:2008055 (2009).

121 Hiss, S., Mielenz, M., Bruckmaier, R. M. & Sauerwein, H. Haptoglobin concentrations in blood and milk after endotoxin challenge and quantification of mammary Hp mRNA

expression. *J. Dairy Sci.* **87**, 3778-3784, doi:10.3168/jds.S0022-0302(04)73516-X (2004).

122 Thielen, M. A. *et al.* Short communication: Cellular localization of haptoglobin mRNA in the experimentally infected bovine mammary gland. *J. Dairy Sci.* **90**, 1215-1219, doi:10.3168/jds.S0022-0302(07)71609-0 (2007).

123 Cooray, R., Waller, K. P. & Venge, P. Haptoglobin comprises about 10% of granule protein extracted from bovine granulocytes isolated from healthy cattle. *Vet. Immunol. Immunopathol.* **119**, 310-315, doi:10.1016/j.vetimm.2007.06.026 (2007).

124 Nagel, R. L. & Gibson, Q. H. The binding of hemoglobin to haptoglobin and its relation to subunit dissociation of hemoglobin. *J. Biol. Chem.* **246**, 69-73 (1971).

125 Graversen, J. H., Madsen, M. & Moestrup, S. K. CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. *Int. J. Biochem. Cell Biol.* **34**, 309-314 (2002).

126 Melamed-Frank, M. *et al.* Structure-function analysis of the antioxidant properties of haptoglobin. *Blood* **98**, 3693-3698 (2001).

127 Eaton, J. W., Brandt, P., Mahoney, J. R. & Lee, J. T., Jr. Haptoglobin: a natural bacteriostat. *Science* **215**, 691-693 (1982).

128 Eckersall, P. D. *et al.* An automated biochemical assay for haptoglobin: Prevention of interference from albumin. *Comparative Haematology International* **9**, 117-124, doi:10.1007/bf02600369 (1999).

129 Morimatsu, M. *et al.* Bovine haptoglobin: single radial immunodiffusion assay of its polymeric forms and dramatic rise in acute-phase sera. *Vet. Immunol. Immunopathol.* **33**, 365-372 (1992).

130 Arredouani, M. S. *et al.* Haptoglobin dampens endotoxin-induced inflammatory effects both in vitro and in vivo. *Immunology* **114**, 263-271, doi:10.1111/j.1365-2567.2004.02071.x (2005).

131 Schaer, C. A., Schoedon, G., Imhof, A., Kurrer, M. O. & Schaer, D. J. Constitutive endocytosis of CD163 mediates hemoglobin-heme uptake and determines the noninflammatory and protective transcriptional response of macrophages to hemoglobin. *Circ. Res.* **99**, 943-950, doi:10.1161/01.RES.0000247067.34173.1b (2006).

132 Saeed, S. A., Ahmad, N. & Ahmed, S. Dual inhibition of cyclooxygenase and lipoxygenase by human haptoglobin: its polymorphism and relation to hemoglobin binding. *Biochem. Biophys. Res. Commun.* **353**, 915-920, doi:10.1016/j.bbrc.2006.12.092 (2007).

133 Bannikov, G. A. *et al.* Serum haptoglobin-matrix metalloproteinase 9 (Hp-MMP 9) complex as a biomarker of systemic inflammation in cattle. *Vet. Immunol. Immunopathol.* **139**, 41-49, doi:10.1016/j.vetimm.2010.08.004 (2011).

134 Bannikov, G. A. *et al.* Biochemical and enzymatic characterization of purified covalent complexes of matrix metalloproteinase-9 and haptoglobin released by bovine granulocytes in vitro. *Am. J. Vet. Res.* **68**, 995-1004, doi:10.2460/ajvr.68.9.995 (2007).

135 Uhlar, C. M. & Whitehead, A. S. Serum amyloid A, the major vertebrate acute-phase reactant. *Eur. J. Biochem.* **265**, 501-523 (1999).

136 Eckersall, P. D. *et al.* Acute phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. *J. Dairy Sci.* **89**, 1488-1501, doi:10.3168/jds.S0022-0302(06)72216-0 (2006).

137 Akerstedt, M., Persson Waller, K. & Sternesjo, A. Haptoglobin and serum amyloid A in relation to the somatic cell count in quarter, cow composite and bulk tank milk samples. *J. Dairy Res.* **74**, 198-203, doi:10.1017/s0022029906002305 (2007).

138 Ceciliani, F. *et al.* The localization and differential expression of Serum Amyloid A in bovine liver and adipose tissue depots. *Vet. Immunol. Immunopathol.* **168**, 35-39, doi:10.1016/j.vetimm.2015.08.004 (2015).

139 Saremi, B. *et al.* Hepatic and extrahepatic expression of serum amyloid A3 during lactation in dairy cows. *J. Dairy Sci.* **96**, 6944-6954, doi:10.3168/jds.2013-6495 (2013).

140 Chapwanya, A., Meade, K. G., Doherty, M. L., Callanan, J. J. & O'Farrelly, C. Endometrial epithelial cells are potent producers of tracheal antimicrobial peptide and serum amyloid A3 gene expression in response to E. coli stimulation. *Vet. Immunol. Immunopathol.* **151**, 157-162, doi:10.1016/j.vetimm.2012.09.042 (2013).

141 Molenaar, A. J. *et al.* The acute-phase protein serum amyloid A3 is expressed in the bovine mammary gland and plays a role in host defence. *Biomarkers* **14**, 26-37, doi:10.1080/13547500902730714 (2009).

142 McDonald, T. L., Larson, M. A., Mack, D. R. & Weber, A. Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. *Vet. Immunol. Immunopathol.* **83**, 203-211 (2001).

143 Mukesh, M., Bionaz, M., Graugnard, D. E., Drackley, J. K. & Loor, J. J. Adipose tissue depots of Holstein cows are immune responsive: inflammatory gene expression in vitro. *Domest. Anim. Endocrinol.* **38**, 168-178, doi:10.1016/j.domaniend.2009.10.001 (2010).

144 Domenech, A., Pares, S., Bach, A. & Aris, A. Mammary serum amyloid A3 activates involution of the mammary gland in dairy cows. *J. Dairy Sci.* **97**, 7595-7605, doi:10.3168/jds.2014-8403 (2014).

145 Badolato, R. *et al.* Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J. Exp. Med.* **180**, 203-209 (1994).

146 Lee, H. Y. *et al.* Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. *Biochem. Biophys. Res. Commun.* **330**, 989-998, doi:10.1016/j.bbrc.2005.03.069 (2005).

147 Larson, M. A., Wei, S. H., Weber, A., Mack, D. R. & McDonald, T. L. Human serum amyloid A3 peptide enhances intestinal MUC3 expression and inhibits EPEC adherence. *Biochem. Biophys. Res. Commun.* **300**, 531-540 (2003).

148 Shah, C., Hari-Dass, R. & Raynes, J. G. Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. *Blood* **108**, 1751-1757, doi:10.1182/blood-2005-11-011932 (2006).

149 Hari-Dass, R., Shah, C., Meyer, D. J. & Raynes, J. G. Serum amyloid A protein binds to outer membrane protein A of gram-negative bacteria. *J. Biol. Chem.* **280**, 18562-18567, doi:10.1074/jbc.M500490200 (2005).

150 Schumann, R. R. *et al.* Structure and function of lipopolysaccharide binding protein. *Science* **249**, 1429-1431 (1990).

151 Bingle, C. D. & Craven, C. J. Meet the relatives: a family of BPI- and LBP-related proteins. *Trends Immunol.* **25**, 53-55 (2004).

152 Khemlani, L. S., Yang, Z. & Bochsler, P. N. Identification and characterization of a bovine lipopolysaccharide-binding protein. *J. Leukoc. Biol.* **56**, 784-791 (1994).

153 Rahman, M. M. *et al.* Lipopolysaccharide-binding protein: Local expression in bovine extrahepatic tissues. *Vet. Immunol. Immunopathol.* **137**, 28-35, doi:10.1016/j.vetimm.2010.04.006 (2010).

154 Horadagoda, N. U. *et al.* Characterisation of bovine lipopolysaccharide binding protein and the in vivo acute phase response to Pasteurella haemolytica Type A. *Vet. Immunol. Immunopathol.* **49**, 61-74 (1995).

155 Schroedl, W., Fuerll, B., Reinhold, P., Krueger, M. & Schuett, C. A novel acute phase marker in cattle: lipopolysaccharide binding protein (LBP). *J. Endotoxin Res.* **7**, 49-52 (2001).

156 Rahman, M. M. *et al.* Expression of alpha1-acid glycoprotein and lipopolysaccharide binding protein in visceral and subcutaneous adipose tissue of dairy cattle. *Vet. J.* **203**, 223-227, doi:10.1016/j.tvjl.2014.12.001 (2015).

157 Zweigner, J., Schumann, R. R. & Weber, J. R. The role of lipopolysaccharide-binding protein in modulating the innate immune response. *Microbes Infect* **8**, 946-952, doi:10.1016/j.micinf.2005.10.006 (2006).

158 Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J. & Mathison, J. C. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* **249**, 1431-1433 (1990).

159 Baldassarre, M., Galeazzi, R., Maggiore, B., Tanfani, F. & Scire, A. Bovine alpha(1)-acid glycoprotein, a thermostable version of its human counterpart: insights from Fourier transform infrared spectroscopy and in silico modelling. *Biochimie* **102**, 19-28, doi:10.1016/j.biochi.2014.02.002 (2014).

160 Tamura, K., Yatsu, T., Itoh, H. & Motoi, Y. Isolation, characterization, and quantitative measurement of serum alpha 1-acid glycoprotein in cattle. *Nihon Juigaku Zasshi* **51**, 987-994 (1989).

161 Ceciliani, F. *et al.* alpha(1)-Acid glycoprotein modulates apoptosis in bovine monocytes. *Vet. Immunol. Immunopathol.* **116**, 145-152, doi:10.1016/j.vetimm.2007.01.006 (2007).

162 Lecchi, C. *et al.* Bovine alpha-1 acid glycoprotein can reduce the chemotaxis of bovine monocytes and modulate CD18 expression. *Vet. Res.* **39**, 50, doi:10.1051/vetres:2008027 (2008).

163 Lecchi, C. *et al.* Alpha(1)-acid glycoprotein modulates phagocytosis and killing of Escherichia coli by bovine polymorphonuclear leucocytes and monocytes. *Vet. J.* **196**, 47-51, doi:10.1016/j.tvjl.2012.07.022 (2013).

164 Miranda-Ribera, A. *et al.* Down-regulatory effect of alpha 1-acid glycoprotein on bovine neutrophil degranulation. *Comp. Immunol. Microbiol. Infect. Dis.* **33**, 291-306, doi:10.1016/j.cimid.2008.10.009 (2010).

165 Rahman, M. M. *et al.* Alpha(1)-acid glycoprotein is contained in bovine neutrophil granules and released after activation. *Vet. Immunol. Immunopathol.* **125**, 71-81, doi:10.1016/j.vetimm.2008.05.010 (2008).

166 Rinaldi, M., Ceciliani, F., Lecchi, C., Moroni, P. & Bannerman, D. D. Differential effects of alpha1-acid glycoprotein on bovine neutrophil respiratory burst activity and IL-8 production. *Vet. Immunol. Immunopathol.* **126**, 199-210, doi:10.1016/j.vetimm.2008.07.001 (2008).

167 Stakauskas, R., Leibold, W., Pieskus, J., Mironova, L. & Schuberth, H. J. Alpha-1-acid glycoprotein inhibits phorbol ester-induced but not Fc-receptor-induced generation of reactive oxygen species in bovine peripheral blood neutrophils. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **52**, 213-218, doi:10.1111/j.1439-0442.2005.00717.x (2005).

168 Tilg, H., Vannier, E., Vachino, G., Dinarello, C. A. & Mier, J. W. Antiinflammatory properties of hepatic acute phase proteins: preferential induction of interleukin 1 (IL-1) receptor antagonist over IL-1 beta synthesis by human peripheral blood mononuclear cells. *J. Exp. Med.* **178**, 1629-1636 (1993).

169 Boutten, A. *et al.* Alpha 1-acid glycoprotein potentiates lipopolysaccharide-induced secretion of interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha by human monocytes and alveolar and peritoneal macrophages. *Eur. J. Immunol.* **22**, 2687-2695, doi:10.1002/eji.1830221032 (1992).

170 Kowsar, R. *et al.* Evidence for a novel, local acute-phase response in the bovine oviduct: progesterone and lipopolysaccharide up-regulate alpha 1-acid-glycoprotein expression in epithelial cells in vitro. *Mol. Reprod. Dev.* **81**, 861-870, doi:10.1002/mrd.22355 (2014).

171 Hochepied, T., Berger, F. G., Baumann, H. & Libert, C. Alpha(1)-acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. *Cytokine Growth Factor Rev.* **14**, 25-34 (2003).

172 Hochepied, T., Van Molle, W., Berger, F. G., Baumann, H. & Libert, C. Involvement of the Acute Phase Protein α1-Acid Glycoprotein in Nonspecific Resistance to a Lethal Gram-negative Infection. *J. Biol. Chem.* **275**, 14903-14909, doi:10.1074/jbc.275.20.14903 (2000).

173 Lecchi, C., Avallone, G., Giurovich, M., Roccabianca, P. & Ceciliani, F. Extra hepatic expression of the acute phase protein alpha 1-acid glycoprotein in normal bovine tissues. *Vet. J.* **180**, 256-258, doi:10.1016/j.tvjl.2007.12.027 (2009).

174 Itoh, H. *et al.* Immunohistochemical localization of alpha 1-acid glycoprotein in liver tissues of bovine fetuses, newborn calves, and sick or healthy adult cattle. *Am. J. Vet. Res.* **58**, 725-728 (1997).

175 Ceciliani, F. *et al.* Differential expression and secretion of alpha1-acid glycoprotein in bovine milk. *J. Dairy Res.* **74**, 374-380, doi:10.1017/s0022029907002646 (2007).

176 Boehmer, J. L. *et al.* Evaluation of protein expression in bovine bronchoalveolar fluid following challenge with Mannheimia haemolytica. *Proteomics* **11**, 3685-3697, doi:10.1002/pmic.201000710 (2011).

177 Ganheim, C. *et al.* The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or Mannheimia haemolytica. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **50**, 183-190 (2003).

178 Hanthorn, C. J. *et al.* Serum concentrations of haptoglobin and haptoglobin-matrix metalloproteinase 9 (Hp-MMP 9) complexes of bovine calves in a bacterial respiratory challenge model. *BMC Vet. Res.* **10**, 285, doi:10.1186/s12917-014-0285-5 (2014).

179 Molina, V. *et al.* Cell-mediated immune response during experimental acute infection with bovine viral diarrhoea virus: evaluation of blood parameters. *Transbound. Emerg. Dis.* **61**, 44-59, doi:10.1111/tbed.12002 (2014).

180 Prohl, A., Schroedl, W., Rhode, H. & Reinhold, P. Acute phase proteins as local biomarkers of respiratory infection in calves. *BMC Vet. Res.* **11**, 167, doi:10.1186/s12917-015-0485-7 (2015).

181 Risalde, M. A. *et al.* Response of proinflammatory and anti-inflammatory cytokines in calves with subclinical bovine viral diarrhea challenged with bovine herpesvirus-1. *Vet. Immunol. Immunopathol.* **144**, 135-143, doi:10.1016/j.vetimm.2011.07.022 (2011).

182 Risalde, M. A. *et al.* Hepatic immune response in calves during acute subclinical infection with bovine viral diarrhoea virus type 1. *Vet. J.* **190**, e110-116, doi:10.1016/j.tvjl.2011.03.003 (2011).

183 Angen, O. *et al.* Respiratory disease in calves: microbiological investigations on trans-tracheally aspirated bronchoalveolar fluid and acute phase protein response. *Vet. Microbiol.* **137**, 165-171, doi:10.1016/j.vetmic.2008.12.024 (2009).

184 Burciaga-Robles, L. O. *et al.* Evaluation of breath biomarkers and serum haptoglobin concentration for diagnosis of bovine respiratory disease in heifers newly arrived at a feedlot. *Am. J. Vet. Res.* **70**, 1291-1298, doi:10.2460/ajvr.70.10.1291 (2009).

185 Idoate, I., Vander Ley, B., Schultz, L. & Heller, M. Acute phase proteins in naturally occurring respiratory disease of feedlot cattle. *Vet. Immunol. Immunopathol.* **163**, 221-226, doi:10.1016/j.vetimm.2014.12.006 (2015).

186 Nikunen, S. *et al.* Association of bovine respiratory disease with clinical status and acute phase proteins in calves. *Comp. Immunol. Microbiol. Infect. Dis.* **30**, 143-151, doi:10.1016/j.cimid.2006.11.004 (2007).

187 Orro, T. *et al.* Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. *Comp. Immunol. Microbiol. Infect. Dis.* **34**, 23-29, doi:10.1016/j.cimid.2009.10.005 (2011).

188 Burciaga-Robles, L. O. *et al.* Effects of exposure to calves persistently infected with bovine viral diarrhea virus type 1b and subsequent infection with Mannheima haemolytica on clinical signs and immune variables: model for bovine respiratory disease via viral and bacterial interaction. *J. Anim. Sci.* **88**, 2166-2178, doi:10.2527/jas.2009-2005 (2010).

189 Richeson, J. T. *et al.* Weaning management of newly received beef calves with or without continuous exposure to a persistently infected bovine viral diarrhea virus pen mate: effects on rectal temperature and serum proinflammatory cytokine and haptoglobin concentrations. *J. Anim. Sci.* **91**, 1400-1408, doi:10.2527/jas.2011-4875 (2013).

190 Kalmus, P. *et al.* Milk haptoglobin, milk amyloid A, and N-acetyl-beta-D-glucosaminidase activity in bovines with naturally occurring clinical mastitis diagnosed with a quantitative PCR test. *J. Dairy Sci.* **96**, 3662-3670, doi:10.3168/jds.2012-6177 (2013).

191 Sadek, K., Saleh, E. & Ayoub, M. Selective, reliable blood and milk bio-markers for diagnosing clinical and subclinical bovine mastitis. *Trop. Anim. Health Prod.* **49**, 431-437, doi:10.1007/s11250-016-1190-7 (2017).

192 Safi, S., Khoshvaghti, A., Jafarzadeh, S. R., Bolourchi, M. & Nowrouzian, I. Acute phase proteins in the diagnosis of bovine subclinical mastitis. *Vet. Clin. Pathol.* **38**, 471-476, doi:10.1111/j.1939-165X.2009.00156.x (2009).

193 Zeng, R., Bequette, B. J., Vinyard, B. T. & Bannerman, D. D. Determination of milk and blood concentrations of lipopolysaccharide-binding protein in cows with naturally acquired subclinical and clinical mastitis. *J. Dairy Sci.* **92**, 980-989, doi:10.3168/jds.2008-1636 (2009).

194 Eckersall, P. D. *et al.* Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet. Rec.* **148**, 35-41 (2001).

195 Jacobsen, S., Niewold, T. A., Kornalijnslijper, E., Toussaint, M. J. & Gruys, E. Kinetics of local and systemic isoforms of serum amyloid A in bovine mastitic milk. *Vet. Immunol. Immunopathol.* **104**, 21-31, doi:10.1016/j.vetimm.2004.09.031 (2005).

196 Burfeind, O., Sannmann, I., Voigtsberger, R. & Heuwieser, W. Receiver operating characteristic curve analysis to determine the diagnostic performance of serum haptoglobin concentration for the diagnosis of acute puerperal metritis in dairy cows. *Anim. Reprod. Sci.* **149**, 145-151, doi:10.1016/j.anireprosci.2014.07.020 (2014).

197 Pohl, A., Burfeind, O. & Heuwieser, W. The associations between postpartum serum haptoglobin concentration and metabolic status, calving difficulties, retained fetal membranes, and metritis. *J. Dairy Sci.* **98**, 4544-4551, doi:10.3168/jds.2014-9181 (2015).

198 Brodzki, P. *et al.* Inflammatory cytokines and acute-phase proteins concentrations in the peripheral blood and uterus of cows that developed endometritis during early postpartum. *Theriogenology* **84**, 11-18, doi:10.1016/j.theriogenology.2015.02.006 (2015).

199 Foley, C. *et al.* Integrated analysis of the local and systemic changes preceding the development of post-partum cytological endometritis. *BMC Genomics* **16**, 811, doi:10.1186/s12864-015-1967-5 (2015).

200 Heidarpour, M., Mohri, M., Fallah-Rad, A. H., Dehghan Shahreza, F. & Mohammadi, M. Acute-phase protein concentration and metabolic status affect the outcome of treatment

in cows with clinical and subclinical endometritis. *Vet. Rec.* **171**, 219, doi:10.1136/vr.100947 (2012).

201 Ganheim, C., Hoglund, J. & Waller, K. P. Acute phase proteins in response to Dictyocaulus viviparus infection in calves. *Acta Vet. Scand.* **45**, 79-86 (2004).

202 Lassen, B., Bangoura, B., Lepik, T. & Orro, T. Systemic acute phase proteins response in calves experimentally infected with Eimeria zuernii. *Vet. Parasitol.* **212**, 140-146, doi:10.1016/j.vetpar.2015.06.024 (2015).

203 Nazifi, S., Razavi, S. M., Esmailnejad, Z. & Gheisari, H. Study on acute phase proteins (haptoglobin, serum amyloid A, fibrinogen, and ceruloplasmin) changes and their diagnostic values in bovine tropical theileriosis. *Parasitol. Res.* **105**, 41-46, doi:10.1007/s00436-009-1360-x (2009).

204 Nazifi, S., Razavi, S. M., Kaviani, F. & Rakhshandehroo, E. Acute phase response in cattle infected with Anaplasma marginale. *Vet. Microbiol.* **155**, 267-271, doi:10.1016/j.vetmic.2011.08.024 (2012).

205 Seppa-Lassila, L. *et al.* Intestinal pathogens, diarrhoea and acute phase proteins in naturally infected dairy calves. *Comp. Immunol. Microbiol. Infect. Dis.* **41**, 10-16, doi:10.1016/j.cimid.2015.05.004 (2015).

206 Baydar, E. & Dabak, M. Serum iron as an indicator of acute inflammation in cattle. *J. Dairy Sci.* **97**, 222-228, doi:10.3168/jds.2013-6939 (2014).

207 Nazifi, S., Ansari-Lari, M., Asadi-Fardaqi, J. & Rezaei, M. The use of receiver operating characteristic (ROC) analysis to assess the diagnostic value of serum amyloid A, haptoglobin and fibrinogen in traumatic reticuloperitonitis in cattle. *Vet. J.* **182**, 315-319, doi:10.1016/j.tvjl.2008.07.002 (2009).

208 Guzelbektes, H. *et al.* Serum amyloid A and haptoglobin concentrations and liver fat percentage in lactating dairy cows with abomasal displacement. *J. Vet. Intern. Med.* **24**, 213-219 (2010).

209 Maden, M., Ozturk, A. S., Bulbul, A., Avci, G. E. & Yazar, E. Acute-phase proteins, oxidative stress and enzyme activities of blood serum and peritoneal fluid in cattle with abomasal displacement. *J. Vet. Intern. Med.* **26**, 1470-1475, doi:10.1111/j.1939-1676.2012.01018.x (2012).

210 Huzzey, J. M., Nydam, D. V., Grant, R. J. & Overton, T. R. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. *J. Dairy Sci.* **94**, 5878-5889, doi:10.3168/jds.2010-3391 (2011).

211 Montagner, P. *et al.* Reduction of liver function delays resumption of postpartum ovarian activity and alters the synthesis of acute phase proteins in dairy cows. *Res. Vet. Sci.* **106**, 84-88, doi:10.1016/j.rvsc.2016.02.015 (2016).

212 Abuelo, A., Gandy, J. C., Neuder, L., Brester, J. & Sordillo, L. M. Short communication: Markers of oxidant status and inflammation relative to the development of claw lesions associated with lameness in early lactation cows. *J. Dairy Sci.* **99**, 5640-5648, doi:10.3168/jds.2015-10707 (2016).

213 Bagga, A., Randhawa, S. S., Sharma, S. & Bansal, B. K. Acute phase response in lame crossbred dairy cattle. *Vet World* **9**, 1204-1208, doi:10.14202/vetworld.2016.1204-1208 (2016).

214 Smith, B. I., Kauffold, J. & Sherman, L. Serum haptoglobin concentrations in dairy cattle with lameness due to claw disorders. *Vet. J.* **186**, 162-165, doi:10.1016/j.tvjl.2009.08.012 (2010).

215 Hepburn, J. J., Arthington, J. D., Hansen, S. L., Spears, J. W. & Knutson, M. D. Technical note: copper chaperone for copper, zinc superoxide dismutase: a potential biomarker for copper status in cattle. *J. Anim. Sci.* **87**, 4161-4166, doi:10.2527/jas.2009-1978 (2009).

216 Arthington, J. D. *et al.* Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. *J. Anim. Sci.* **91**, 1831-1837, doi:10.2527/jas.2012-5724 (2013).

217 Richeson, J. T. *et al.* Dexamethasone treatment differentially alters viral shedding and the antibody and acute phase protein response after multivalent respiratory vaccination in beef steers. *J. Anim. Sci.* **94**, 3501-3509, doi:10.2527/jas.2016-0572 (2016).

218 Rodrigues, M. C. *et al.* Effects of vaccination against respiratory pathogens on feed intake, metabolic, and inflammatory responses in beef heifers. *J. Anim. Sci.* **93**, 4443-4452, doi:10.2527/jas.2015-9277 (2015).

219 Roberts, S. L. *et al.* Effect of surgical castration with or without oral meloxicam on the acute inflammatory response in yearling beef bulls. *J. Anim. Sci.* **93**, 4123-4131, doi:10.2527/jas.2015-9160 (2015).

220 Ganheim, C., Alenius, S. & Persson Waller, K. Acute phase proteins as indicators of calf herd health. *Vet. J.* **173**, 645-651, doi:10.1016/j.tvjl.2006.01.011 (2007).

221 Gifford, C. A. *et al.* Growth and Development Symposium: Impacts of inflammation on cattle growth and carcass merit. *J. Anim. Sci.* **90**, 1438-1451, doi:10.2527/jas.2011-4846 (2012).

222 Holland, B. P. *et al.* Effectiveness of sorting calves with high risk of developing bovine respiratory disease on the basis of serum haptoglobin concentration at the time of arrival at a feedlot. *Am. J. Vet. Res.* **72**, 1349-1360, doi:10.2460/ajvr.72.10.1349 (2011).

223 Jawor, P., Stefaniak, T. & Mee, J. F. Immune and inflammatory biomarkers in cases of bovine perinatal mortality with and without infection in utero. *J. Dairy Sci.* **100**, 1408-1416, doi:10.3168/jds.2016-11825 (2017).

224 Simoes, P. B. A. *et al.* Pilot study into milk haptoglobin as an indicator of udder health in heifers after calving. *Res. Vet. Sci.*, doi:10.1016/j.rvsc.2017.05.024 (2017).

225 Svensson, C., Liberg, P. & Hultgren, J. Evaluating the efficacy of serum haptoglobin concentration as an indicator of respiratory-tract disease in dairy calves. *Vet. J.* **174**, 288-294, doi:10.1016/j.tvjl.2006.07.009 (2007).

226 Dowling, A. *et al.* Experimental induction of pneumonic pasteurellosis in calves by intratracheal infection with Pasteurella multocida biotype A:3. *Res. Vet. Sci.* **73**, 37-44 (2002).

227 Heegaard, P. M. *et al.* The acute phase response of haptoglobin and serum amyloid A (SAA) in cattle undergoing experimental infection with bovine respiratory syncytial virus. *Vet. Immunol. Immunopathol.* **77**, 151-159 (2000).

228 Godson, D. L. *et al.* Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. *Vet. Immunol. Immunopathol.* **51**, 277-292 (1996).

229 Gronlund, U., Hulten, C., Eckersall, P. D., Hogarth, C. & Persson Waller, K. Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced Staphylococcus aureus mastitis. *J. Dairy Res.* **70**, 379-386 (2003).

230 Kim, Y., Atalla, H., Mallard, B., Robert, C. & Karrow, N. Changes in Holstein cow milk and serum proteins during intramammary infection with three different strains of Staphylococcus aureus. *BMC Vet. Res.* **7**, 51, doi:10.1186/1746-6148-7-51 (2011).

231 Pedersen, L. H. *et al.* Early pathogenesis and inflammatory response in experimental bovine mastitis due to Streptococcus uberis. *J. Comp. Pathol.* **128**, 156-164 (2003).

232 Suojala, L., Orro, T., Jarvinen, H., Saatsi, J. & Pyorala, S. Acute phase response in two consecutive experimentally induced E. coli intramammary infections in dairy cows. *Acta Vet. Scand.* **50**, 18, doi:10.1186/1751-0147-50-18 (2008).

233 Carter, J. N. *et al.* Relationship of vitamin E supplementation and antimicrobial treatment with acute-phase protein responses in cattle affected by naturally acquired respiratory tract disease. *Am. J. Vet. Res.* **63**, 1111-1117 (2002).

234 Berry, B. A. *et al.* Effects of dietary energy and starch concentrations for newly received feedlot calves: II. Acute-phase protein response. *J. Anim. Sci.* **82**, 845-850 (2004).

235 Humblet, M. F., Coghe, J., Lekeux, P. & Godeau, J. M. Acute phase proteins assessment for an early selection of treatments in growing calves suffering from bronchopneumonia under field conditions. *Res. Vet. Sci.* **77**, 41-47, doi:10.1016/j.rvsc.2004.02.009 (2004).

236 Young, C. R. *et al.* Serum haptoglobin concentrations in a population of feedlot cattle. *Am. J. Vet. Res.* **57**, 138-141 (1996).

237 Wittum, T. E. *et al.* Haptoglobin response to clinical respiratory tract disease in feedlot cattle. *Am. J. Vet. Res.* **57**, 646-649 (1996).

238 Abdallah, A., Hewson, J., Francoz, D., Selim, H. & Buczinski, S. Systematic Review of the Diagnostic Accuracy of Haptoglobin, Serum Amyloid A, and Fibrinogen versus Clinical Reference Standards for the Diagnosis of Bovine Respiratory Disease. *J. Vet. Intern. Med.* **30**, 1356-1368, doi:10.1111/jvim.13975 (2016).

239 Gray, M. L., Young, C., Stanker, L. H. & Bounous, D. I. Measurement of serum haptoglobin in neonatal farm-raised and bob veal calves using two immunoassay methods. *Vet. Clin. Pathol.* **25**, 38-42 (1996).

240 Hirvonen, J., Hietakorpi, S. & Saloniemi, H. Acute phase response in emergency slaughtered dairy cows. *Meat Sci* **46**, 249-257 (1997).

241 Tourlomoussis, P., Eckersall, P. D., Waterson, M. M. & Buncic, S. A comparison of acute phase protein measurements and meat inspection findings in cattle. *Foodborne Pathog. Dis.* **1**, 281-290, doi:10.1089/fpd.2004.1.281 (2004).

242 Piccione, G., Caola, G. & Refinetti, R. Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiol.* **3**, 7, doi:10.1186/1472-6793-3-7 (2003).

243 Bitman, J., Lefcourt, A., Wood, D. L. & Stroud, B. Circadian and ultradian temperature rhythms of lactating dairy cows. *J. Dairy Sci.* **67**, 1014-1023, doi:10.3168/jds.S0022-0302(84)81400-9 (1984).

244 Lefcourt, A. M. & Adams, W. R. Radiotelemetric measurement of body temperature in feedlot steers during winter. *J. Anim. Sci.* **76**, 1830-1837 (1998).

245 Lefcourt, A. M. & Schmidtmann, E. T. Body temperature of dry cows on pasture: environmental and behavioral effects. *J. Dairy Sci.* **72**, 3040-3049, doi:10.3168/jds.S0022-0302(89)79457-1 (1989).

246 Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L. & Bitman, J. Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. *Domest. Anim. Endocrinol.* **16**, 41-55 (1999).

247 Bewley, J. M., Grott, M. W., Einstein, M. E. & Schutz, M. M. Impact of intake water temperatures on reticular temperatures of lactating dairy cows. *J. Dairy Sci.* **91**, 3880-3887, doi:10.3168/jds.2008-1159 (2008).

248 Suthar, V. S., Burfeind, O., Patel, J. S., Dhami, A. J. & Heuwieser, W. Body temperature around induced estrus in dairy cows. *J. Dairy Sci.* **94**, 2368-2373, doi:10.3168/jds.2010-3858 (2011).

249 Suthar, V. *et al.* Factors associated with body temperature of healthy Holstein dairy cows during the first 10 days in milk. *J. Dairy Res.* **79**, 135-142, doi:10.1017/s0022029911000896 (2012).

250 Adams, A. E., Olea-Popelka, F. J. & Roman-Muniz, I. N. Using temperature-sensing reticular boluses to aid in the detection of production diseases in dairy cows. *J. Dairy Sci.* **96**, 1549-1555, doi:10.3168/jds.2012-5822 (2013).

251 Liang, D. *et al.* Influence of breed, milk production, season, and ambient temperature on dairy cow reticulorumen temperature. *J. Dairy Sci.* **96**, 5072-5081, doi:10.3168/jds.2012-6537 (2013).

252 Burfeind, O., Suthar, V. S. & Heuwieser, W. Effect of heat stress on body temperature in healthy early postpartum dairy cows. *Theriogenology* **78**, 2031-2038, doi:10.1016/j.theriogenology.2012.07.024 (2012).

253 Suthar, V., Burfeind, O., Maeder, B. & Heuwieser, W. Agreement between rectal and vaginal temperature measured with temperature loggers in dairy cows. *J. Dairy Res.* **80**, 240-245, doi:10.1017/s0022029913000071 (2013).

254 Lee, C. N., Gebremedhin, K. G., Parkhurst, A. & Hillman, P. E. Placement of temperature probe in bovine vagina for continuous measurement of core-body temperature. *Int. J. Biometeorol.* **59**, 1201-1205, doi:10.1007/s00484-014-0931-4 (2015).

255 Bewley, J. M., Einstein, M. E., Grott, M. W. & Schutz, M. M. Comparison of reticular and rectal core body temperatures in lactating dairy cows. *J. Dairy Sci.* **91**, 4661-4672, doi:10.3168/jds.2007-0835 (2008).

256 AlZahal, O., Steele, M. A., Valdes, E. V. & McBride, B. W. Technical note: the use of a telemetric system to continuously monitor ruminal temperature and to predict ruminal pH in cattle. *J. Dairy Sci.* **92**, 5697-5701, doi:10.3168/jds.2009-2220 (2009).

257 Lefcourt, A. M., Bitman, J., Wood, D. L. & Stroud, B. Radiotelemetry system for continuously monitoring temperature in cows. *J. Dairy Sci.* **69**, 237-242, doi:10.3168/jds.S0022-0302(86)80391-5 (1986).

258 Bergen, R. D. & Kennedy, A. D. Relationship between vaginal and tympanic membrane temperature in beef heifers. *Canadian Journal of Animal Science* **80**, 515-518, doi:10.4141/A00-033 (2000).

259 Hahn, G. L., Eigenberg, R. A., Nienaber, J. A. & Littledike, E. T. Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. *J. Anim. Sci.* **68**, 2658-2665 (1990).

260 Vickers, L. A. *et al.* Technical note: Comparison of rectal and vaginal temperatures in lactating dairy cows. *J. Dairy Sci.* **93**, 5246-5251, doi:10.3168/jds.2010-3388 (2010).

261 Sannmann, I., Burfeind, O., Voigtsberger, R. & Heuwieser, W. Comparison of two monitoring and treatment strategies for cows with acute puerperal metritis. *Theriogenology* **79**, 961-969, doi:10.1016/j.theriogenology.2013.01.016 (2013).

262 Smith, B. I. & Risco, C. A. Management of periparturient disorders in dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.* **21**, 503-521, doi:10.1016/j.cvfa.2005.02.007 (2005).

263 Benzaquen, M. E. *et al.* Rectal Temperature, Calving-Related Factors, and the Incidence of Puerperal Metritis in Postpartum Dairy Cows. *J. Dairy Sci.* **90**, 2804-2814, doi:10.3168/jds.2006-482.

264 Duff, G. C. & Galyean, M. L. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *J. Anim. Sci.* **85**, 823-840, doi:10.2527/jas.2006-501 (2007).

265 Toaff-Rosenstein, R. L., Gershwin, L. J. & Tucker, C. B. Fever, feeding, and grooming behavior around peak clinical signs in bovine respiratory disease. *J. Anim. Sci.* **94**, 3918-3932, doi:10.2527/jas.2016-0346 (2016).

266 Avra, T. D. *et al.* A retrospective analysis of risk factors associated with bovine respiratory disease treatment failure in feedlot cattle. *J. Anim. Sci.* **95**, 1521-1527, doi:10.2527/jas.2016.1254 (2017).

267 Theurer, M. E., White, B. J., Larson, R. L., Holstein, K. K. & Amrine, D. E. Relationship between rectal temperature at first treatment for bovine respiratory disease complex in feedlot calves and the probability of not finishing the production cycle. *J. Am. Vet. Med. Assoc.* **245**, 1279-1285, doi:10.2460/javma.245.11.1279 (2014).

268 Burfeind, O., von Keyserlingk, M. A., Weary, D. M., Veira, D. M. & Heuwieser, W. Short communication: repeatability of measures of rectal temperature in dairy cows. *J. Dairy Sci.* **93**, 624-627, doi:10.3168/jds.2009-2689 (2010).

269 Baldwin, H. A., Depp, S. W., Koelle, A. R. & Freyman, R. W. Electronic animal identification and temperature monitoring. *Proc. Annu. Meet. U. S. Anim. Health Assoc.*, 141-152d (1973).

270 Seawright, G. L. Remote temperature monitoring in animal health management. *Proc. Annu. Meet. U. S. Anim. Health Assoc.*, 214-230 (1976).

271 Maxwell, B. M., Brunell, M. K., Olsen, C. H. & Bentzel, D. E. Comparison of Digital Rectal and Microchip Transponder Thermometry in Ferrets (Mustela putorius furo). *J Am Assoc Lab Anim Sci* **55**, 331-335 (2016).

272 Quimby, J. M., Olea-Popelka, F. & Lappin, M. R. Comparison of digital rectal and microchip transponder thermometry in cats. *J Am Assoc Lab Anim Sci* **48**, 402-404 (2009).

273 Jara, A. L. *et al.* Comparison of Microchip Transponder and Noncontact Infrared Thermometry with Rectal Thermometry in Domestic Swine (Sus scrofa domestica). *J Am Assoc Lab Anim Sci* **55**, 588-593 (2016).

274 Goodwin, S. Comparison of Body Temperatures of Goats, Horses, and Sheep Measured With a Tympanic Infrared Thermometer, an Implantable Microchip Transponder, and a Rectal Thermometer. *Contemp Top Lab Anim Sci* **37**, 51-55 (1998).

275 Brunell, M. K. Comparison of noncontact infrared thermometry and 3 commercial subcutaneous temperature transponding microchips with rectal thermometry in rhesus macaques (Macaca mulatta). *J Am Assoc Lab Anim Sci* **51**, 479-484 (2012).

276 Rey, B. *et al.* Microchip transponder thermometry for monitoring core body temperature of antelope during capture. *J. Therm. Biol.* **55**, 47-53, doi:10.1016/j.jtherbio.2015.11.010 (2016).

277 Lee, Y. *et al.* Body Temperature Monitoring Using Subcutaneously Implanted Thermo-loggers from Holstein Steers. *Asian-Australas J Anim Sci* **29**, 299-306, doi:10.5713/ajas.15.0353 (2016).

278 Grissett, G. P., White, B. J., Anderson, D. E., Larson, R. E. & Miesner, M. D. Effect of ambient temperature on viral replication and serum antibody titers following administration of a commercial intranasal modified-live infectious bovine rhinotracheitis-parainfluenza-3 virus vaccine to beef cattle housed in high- and moderate-ambient temperature environments. *Am. J. Vet. Res.* **75**, 1076-1082, doi:10.2460/ajvr.75.12.1076 (2014).

279 Greer, R. J., Cohn, L. A., Dodam, J. R., Wagner-Mann, C. C. & Mann, F. A. Comparison of three methods of temperature measurement in hypothermic, euthermic, and hyperthermic dogs. *J. Am. Vet. Med. Assoc.* **230**, 1841-1848, doi:10.2460/javma.230.12.1841 (2007).

280 Robinson, T. R. *et al.* Comparison of temperature readings from a percutaneous thermal sensing microchip with temperature readings from a digital rectal thermometer in equids. *J. Am. Vet. Med. Assoc.* **233**, 613-617, doi:10.2460/javma.233.4.613 (2008).

281 Nogami, H., Okada, H., Miyamoto, T., Maeda, R. & Itoh, T. Wearable and compact wireless sensor nodes for measuring the temperature of the base of a calf's tail *Sensors and Materials* **25**, 577-582 (2013).

282 Miura, R. *et al.* Estrous detection by monitoring ventral tail base surface temperature using a wearable wireless sensor in cattle. *Anim. Reprod. Sci.* **180**, 50-57, doi:10.1016/j.anireprosci.2017.03.002 (2017).

283 Kyle, B. L., Kennedy, A. D. & Small, J. A. Measurement of vaginal temperature by radiotelemetry for the prediction of estrus in beef cows. *Theriogenology* **49**, 1437-1449 (1998).

284 Redden, K. D., Kennedy, A. D., Ingalls, J. R. & Gilson, T. L. Detection of estrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. *J. Dairy Sci.* **76**, 713-721, doi:10.3168/jds.S0022-0302(93)77394-4 (1993).

285 Clapper, J. A., Ottobre, J. S., Ottobre, A. C. & Zartman, D. L. Estrual rise in body temperature in the bovine I. Temporal relationships with serum patterns of reproductive hormones. *Anim. Reprod. Sci.* **23**, 89-98, doi:http://dx.doi.org/10.1016/0378-4320(90)90051-G (1990).

286 Mosher, M. D., Ottobre, J. S., Haibel, G. K. & Zartman, D. L. Estrual rise in body temperature in the bovine II. The temporal relationship with ovulation. *Anim. Reprod. Sci.* **23**, 99-107, doi:http://dx.doi.org/10.1016/0378-4320(90)90052-H (1990).

287 Rajamahendran, R., Robinson, J., Desbottes, S. & Walton, J. S. Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels, and ovulation in dairy cows. *Theriogenology* **31**, 1173-1182 (1989).

288 Fisher, A. D., Morton, R., Dempsey, J. M., Henshall, J. M. & Hill, J. R. Evaluation of a new approach for the estimation of the time of the LH surge in dairy cows using vaginal temperature and electrodeless conductivity measurements. *Theriogenology* **70**, 1065-1074, doi:10.1016/j.theriogenology.2008.06.023 (2008).

289 Fordham, D. P., McCarthy, T. T. & Rowlinson, P. An evaluation of milk temperature measurement for detecting oestrus in dairy cattle. II. Variations in body and milk temperature associated with oestrus. *Vet. Res. Commun.* **11**, 381-391 (1987).

290 Fordham, D. P., McCarthy, T. T. & Rowlinson, P. An evaluation of milk temperature measurement for detecting oestrus in dairy cattle. I. Factors affecting measurement of milk temperature. *Vet. Res. Commun.* **11**, 367-379 (1987).

291 Fordham, D. P., Rowlinson, P. & McCarthy, T. T. Oestrus detection in dairy cows by milk temperature measurement. *Res. Vet. Sci.* **44**, 366-374 (1988).

292 Sakatani, M., Takahashi, M. & Takenouchi, N. The efficiency of vaginal temperature measurement for detection of estrus in Japanese Black cows. *J Reprod Dev* **62**, 201-207, doi:10.1262/jrd.2015-095 (2016).

293 Aoki, M., Kimura, K. & Suzuki, O. Predicting time of parturition from changing vaginal temperature measured by data-logging apparatus in beef cows with twin fetuses. *Anim. Reprod. Sci.* **86**, 1-12, doi:10.1016/j.anireprosci.2004.04.046 (2005).

294 McCorkell, R., Wynne-Edwards, K., Windeyer, C. & Schaefer, A. Limited efficacy of Fever Tag((R)) temperature sensing ear tags in calves with naturally occurring bovine respiratory disease or induced bovine viral diarrhea virus infection. *Can. Vet. J.* **55**, 688-690 (2014).

295 Mahendran, S. A., Booth, R., Bell, N. J. & Burge, M. Randomised positive control trial of NSAID and antimicrobial treatment for calf fever caused by pneumonia. *Vet. Rec.* **181**, 45, doi:10.1136/vr.104057 (2017).

296 Antonini, C. *et al.* In vivo mechanical and in vitro electromagnetic side-effects of a ruminal transponder in cattle. *J. Anim. Sci.* **84**, 3133-3142, doi:10.2527/jas.2006-136 (2006).

297 Carne, S., Caja, G., Ghirardi, J. J. & Salama, A. A. Modeling the retention of rumen boluses for the electronic identification of goats. *J. Dairy Sci.* **94**, 716-726, doi:10.3168/jds.2010-3210 (2011).

298 Ghirardi, J. J., Caja, G., Garin, D., Casellas, J. & Hernandez-Jover, M. Evaluation of the retention of electronic identification boluses in the forestomachs of cattle. *J. Anim. Sci.* **84**, 2260-2268, doi:10.2527/jas.2005-758 (2006).

299 Ghirardi, J. J. *et al.* Retention of different sizes of electronic identification boluses in the forestomachs of sheep. *J. Anim. Sci.* **84**, 2865-2872, doi:10.2527/jas.2006-157 (2006).

300 Rose-Dye, T. K. *et al.* Rumen temperature change monitored with remote rumen temperature boluses after challenges with bovine viral diarrhea virus and Mannheimia haemolytica. *J. Anim. Sci.* **89**, 1193-1200, doi:10.2527/jas.2010-3051 (2011).

301 Timsit, E., Assie, S., Quiniou, R., Seegers, H. & Bareille, N. Early detection of bovine respiratory disease in young bulls using reticulo-rumen temperature boluses. *Vet. J.* **190**, 136-142, doi:10.1016/j.tvjl.2010.09.012 (2011).

302 Timsit, E., Bareille, N., Seegers, H., Lehebel, A. & Assie, S. Visually undetected fever episodes in newly received beef bulls at a fattening operation: occurrence, duration, and impact on performance. *J. Anim. Sci.* **89**, 4272-4280, doi:10.2527/jas.2011-3892 (2011).

303 Sato, S. *et al.* A radio transmission pH measurement system for continuous evaluation of fluid pH in the rumen of cows. *Vet. Res. Commun.* **36**, 85-89, doi:10.1007/s11259-012-9518-x (2012).

304 Kimura, A. *et al.* Relationship between pH and temperature in the ruminal fluid of cows, based on a radio-transmission pH-measurement system. *J. Vet. Med. Sci.* **74**, 1023-1028 (2012).

305 Mohammed, R., Hunerberg, M., McAllister, T. A. & Beauchemin, K. A. Characterization of ruminal temperature and its relationship with ruminal pH in beef heifers fed growing and finishing diets. *J. Anim. Sci.* **92**, 4650-4660, doi:10.2527/jas.2014-7859 (2014).

306 Alzahal, O. *et al.* The use of a radiotelemetric ruminal bolus to detect body temperature changes in lactating dairy cattle. *J. Dairy Sci.* **94**, 3568-3574, doi:10.3168/jds.2010-3944 (2011).

307 Cooper-Prado, M. J., Long, N. M., Wright, E. C., Goad, C. L. & Wettemann, R. P. Relationship of ruminal temperature with parturition and estrus of beef cows. *J. Anim. Sci.* **89**, 1020-1027, doi:10.2527/jas.2010-3434 (2011).

308 Gershon-Cohen, J. & Haberman, J. D. THERMOGRAPHY. *Radiology* **82**, 280-285, doi:10.1148/82.2.280 (1964).

309 Tucker, C. B. *et al.* Effect of a cooling gel on pain sensitivity and healing of hot-iron cattle brands. *J. Anim. Sci.* **92**, 5666-5673, doi:10.2527/jas.2014-7860 (2014).

310 Unruh, E. M. *et al.* Evaluation of infrared thermography as a diagnostic tool to predict heat stress events in feedlot cattle. *Am. J. Vet. Res.* **78**, 771-777, doi:10.2460/ajvr.78.7.771 (2017).

311 Coetzee, J. F. Assessment and management of pain associated with castration in cattle. *Vet. Clin. North Am. Food Anim. Pract.* **29**, 75-101, doi:10.1016/j.cvfa.2012.11.002 (2013).

312 Dockweiler, J. C. *et al.* Effect of castration method on neurohormonal and electroencephalographic stress indicators in Holstein calves of different ages. *J. Dairy Sci.* **96**, 4340-4354, doi:10.3168/jds.2012-6274 (2013).

313 Glynn, H. D. *et al.* The pharmacokinetics and effects of meloxicam, gabapentin, and flunixin in postweaning dairy calves following dehorning with local anesthesia. *J. Vet. Pharmacol. Ther.* **36**, 550-561, doi:10.1111/jvp.12042 (2013).

314 Oliveira, F. C. *et al.* Chemical castration in cattle with intratesticular injection of sodium chloride: Effects on stress and inflammatory markers. *Theriogenology* **90**, 114-119, doi:10.1016/j.theriogenology.2016.12.001 (2017).

315 Stewart, M. *et al.* Effects of local anesthetic and a nonsteroidal antiinflammatory drug on pain responses of dairy calves to hot-iron dehorning. *J. Dairy Sci.* **92**, 1512-1519, doi:10.3168/jds.2008-1578 (2009).

316 Stewart, M., Verkerk, G. A., Stafford, K. J., Schaefer, A. L. & Webster, J. R. Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *J. Dairy Sci.* **93**, 3602-3609, doi:10.3168/jds.2010-3114 (2010).

317 Stock, M. L. *et al.* The effects of firocoxib on cautery disbudding pain and stress responses in preweaned dairy calves. *J. Dairy Sci.* **98**, 6058-6069, doi:10.3168/jds.2014-8877 (2015).

318 George, W. D., Godfrey, R. W., Ketring, R. C., Vinson, M. C. & Willard, S. T. Relationship among eye and muzzle temperatures measured using digital infrared thermal imaging and vaginal and rectal temperatures in hair sheep and cattle. *J. Anim. Sci.* **92**, 4949-4955, doi:10.2527/jas.2014-8087 (2014).

319 Byrne, D. T., Berry, D. P., Esmonde, H. & McHugh, N. Temporal, spatial, inter-, and intra-cow repeatability of thermal imaging. *J. Anim. Sci.* **95**, 970-979, doi:10.2527/jas.2016.1005 (2017).

320 Hoffmann, G. *et al.* Monitoring the body temperature of cows and calves using video recordings from an infrared thermography camera. *Vet. Res. Commun.* **37**, 91-99, doi:10.1007/s11259-012-9549-3 (2013).

321 Salles, M. S. *et al.* Mapping the body surface temperature of cattle by infrared thermography. *J. Therm. Biol.* **62**, 63-69, doi:10.1016/j.jtherbio.2016.10.003 (2016).

322 McCafferty, D. J. The value of infrared thermography for research on mammals: previous applications and future directions. *Mamm. Rev.* **37**, 207-223, doi:10.1111/j.1365-2907.2007.00111.x (2007).

323 Church, J. S. *et al.* Influence of environmental factors on infrared eye temperature measurements in cattle. *Res. Vet. Sci.* **96**, 220-226, doi:10.1016/j.rvsc.2013.11.006 (2014).

324 Hurnik, J. F., Webster, A. B. & DeBoer, S. An investigation of skin temperature differentials in relation to estrus in dairy cattle using a thermal infrared scanning technique. *J. Anim. Sci.* **61**, 1095-1102 (1985).

325 Talukder, S. *et al.* Infrared technology for estrus detection and as a predictor of time of ovulation in dairy cows in a pasture-based system. *Theriogenology* **81**, 925-935, doi:10.1016/j.theriogenology.2014.01.009 (2014).

326 Talukder, S., Thomson, P. C., Kerrisk, K. L., Clark, C. E. & Celi, P. Evaluation of infrared thermography body temperature and collar-mounted accelerometer and acoustic technology for predicting time of ovulation of cows in a pasture-based system. *Theriogenology* **83**, 739-748, doi:10.1016/j.theriogenology.2014.11.005 (2015).

327 Hovinen, M. *et al.* Detection of Clinical Mastitis with the Help of a Thermal Camera. *J. Dairy Sci.* **91**, 4592-4598, doi:http://dx.doi.org/10.3168/jds.2008-1218 (2008).

328 Pezeshki, A. *et al.* Variation of inflammatory dynamics and mediators in primiparous cows after intramammary challenge with Escherichia coli. *Vet. Res.* **42**, 15, doi:10.1186/1297-9716-42-15 (2011).

329 Metzner, M., Sauter-Louis, C., Seemueller, A., Petzl, W. & Klee, W. Infrared thermography of the udder surface of dairy cattle: characteristics, methods, and correlation with rectal temperature. *Vet. J.* **199**, 57-62, doi:10.1016/j.tvjl.2013.10.030 (2014).

330 Metzner, M., Sauter-Louis, C., Seemueller, A., Petzl, W. & Zerbe, H. Infrared thermography of the udder after experimentally induced Escherichia coli mastitis in cows. *Vet. J.* **204**, 360-362, doi:10.1016/j.tvjl.2015.04.013 (2015).

331 Colak, A. *et al.* Short Communication: Early Detection of Mastitis Using Infrared Thermography in Dairy Cows1. *J. Dairy Sci.* **91**, 4244-4248, doi:http://dx.doi.org/10.3168/jds.2008-1258 (2008).

332 Polat, B. *et al.* Sensitivity and specificity of infrared thermography in detection of subclinical mastitis in dairy cows. *J. Dairy Sci.* **93**, 3525-3532, doi:http://dx.doi.org/10.3168/jds.2009-2807 (2010).

333 Bortolami, A. *et al.* Evaluation of the udder health status in subclinical mastitis affected dairy cows through bacteriological culture, somatic cell count and thermographic imaging. *Pol. J. Vet. Sci.* **18**, 799-805, doi:10.1515/pjvs-2015-0104 (2015).

334 Schaefer, A. L. *et al.* Early detection and prediction of infection using infrared thermography. *Canadian Journal of Animal Science* **84**, 73-80, doi:10.4141/A02-104 (2004).

335 Schaefer, A. L. *et al.* The non-invasive and automated detection of bovine respiratory disease onset in receiver calves using infrared thermography. *Res. Vet. Sci.* **93**, 928-935, doi:10.1016/j.rvsc.2011.09.021 (2012).

336 Schaefer, A. L. *et al.* The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. *Res. Vet. Sci.* **83**, 376-384, doi:10.1016/j.rvsc.2007.01.008 (2007).

337 Fraser, B. C. *et al.* Associations of various physical and blood analysis variables with experimentally induced Mycoplasma bovis pneumonia in calves. *Am. J. Vet. Res.* **75**, 200-207, doi:10.2460/ajvr.75.2.200 (2014).

338 Caldwell, M., Passler, T., Purohit, R. C., Pascoe, D. & Wolfe, D. F. Diagnosis of lumbosacral diskospondylosis in a bucking bull assisted by high-definition thermal and nuclear scintigraphic imaging. *J. Am. Vet. Med. Assoc.* **250**, 554-560, doi:10.2460/javma.250.5.554 (2017).

339 Cockcroft, P. D., Henson, F. M. & Parker, C. Thermography of a septic metatarsophalangeal joint in a heifer. *Vet. Rec.* **146**, 258-260 (2000).

340 Wilhelm, K., Wilhelm, J. & Furll, M. Use of thermography to monitor sole haemorrhages and temperature distribution over the claws of dairy cattle. *Vet. Rec.* **176**, 146, doi:10.1136/vr.101547 (2015).

341 Oikonomou, G., Trojacanec, P., Ganda, E. K., Bicalho, M. L. & Bicalho, R. C. Association of digital cushion thickness with sole temperature measured with the use of infrared thermography. *J. Dairy Sci.* **97**, 4208-4215, doi:10.3168/jds.2013-7534 (2014).

342 Alsaaod, M. & Büscher, W. Detection of hoof lesions using digital infrared thermography in dairy cows. *J. Dairy Sci.* **95**, 735-742, doi:http://dx.doi.org/10.3168/jds.2011-4762 (2012).

343 Nikkhah, A. *et al.* Short communication: infrared thermography and visual examination of hooves of dairy cows in two stages of lactation. *J. Dairy Sci.* **88**, 2749-2753, doi:10.3168/jds.S0022-0302(05)72954-4 (2005).

344 Stokes, J. E., Leach, K. A., Main, D. C. J. & Whay, H. R. An investigation into the use of infrared thermography (IRT) as a rapid diagnostic tool for foot lesions in dairy cattle. *The Veterinary Journal* **193**, 674-678, doi:http://dx.doi.org/10.1016/j.tvjl.2012.06.052 (2012).

345 Wood, S., Lin, Y., Knowles, T. G. & Main, D. C. Infrared thermometry for lesion monitoring in cattle lameness. *Vet. Rec.* **176**, 308, doi:10.1136/vr.102571 (2015).

346 Alsaaod, M. *et al.* A field trial of infrared thermography as a non-invasive diagnostic tool for early detection of digital dermatitis in dairy cows. *Vet. J.* **199**, 281-285, doi:10.1016/j.tvjl.2013.11.028 (2014).

347 Bowser, D. in *Feedlot* Vol. VII (1999).

348 Wilkins, D. in *Progressive Dairyman* (2012).

349 Sowell, B. F., Bowman, J. G. P., Branine, M. E. & Hubbert, M. E. Radio frequency technology to measure feeding behavior and health of feedlot steers. *Appl. Anim. Behav. Sci.* **59**, 277-284, doi:http://dx.doi.org/10.1016/S0168-1591(98)00110-5 (1998).

350 Gibb, D. J., McAllister, T. A., Huisma, C. & Wiedmeier, R. D. Bunk attendance of feedlot cattle monitored with radio frequency technology. *Canadian Journal of Animal Science* **78**, 707-710, doi:10.4141/A98-032 (1998).

351 Mendes, E. D., Carstens, G. E., Tedeschi, L. O., Pinchak, W. E. & Friend, T. H. Validation of a system for monitoring feeding behavior in beef cattle. *J. Anim. Sci.* **89**, 2904-2910, doi:10.2527/jas.2010-3489 (2011).

352 Quimby, W. F. *et al.* Application of feeding behaviour to predict morbidity of newly received calves in a commercial feedlot. *Canadian Journal of Animal Science* **81**, 315-320, doi:10.4141/A00-098 (2001).

353 Urton, G., von Keyserlingk, M. A. & Weary, D. M. Feeding behavior identifies dairy cows at risk for metritis. *J. Dairy Sci.* **88**, 2843-2849, doi:10.3168/jds.S0022-0302(05)72965-9 (2005).

354 Lancaster, P. A., Carstens, G. E., Ribeiro, F. R., Tedeschi, L. O. & Crews, D. H., Jr. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. *J. Anim. Sci.* **87**, 1528-1539, doi:10.2527/jas.2008-1352 (2009).

355 Chapinal, N., Veira, D. M., Weary, D. M. & von Keyserlingk, M. A. Technical note: validation of a system for monitoring individual feeding and drinking behavior and intake in group-housed cattle. *J. Dairy Sci.* **90**, 5732-5736, doi:10.3168/jds.2007-0331 (2007).

356 DeVries, T. J., von Keyserlingk, M. A., Weary, D. M. & Beauchemin, K. A. Technical note: Validation of a system for monitoring feeding behavior of dairy cows. *J. Dairy Sci.* **86**, 3571-3574, doi:10.3168/jds.S0022-0302(03)73962-9 (2003).

357 Wang, Z. *et al.* Test duration for growth, feed intake, and feed efficiency in beef cattle using the GrowSafe System. *J. Anim. Sci.* **84**, 2289-2298, doi:10.2527/jas.2005-715 (2006).

358 Schwartzkopf-Genswein, K. S., Atwood, S. & McAllister, T. A. Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. *Appl. Anim. Behav. Sci.* **76**, 179-188, doi:http://dx.doi.org/10.1016/S0168-1591(02)00009-6 (2002).

359 Schwartzkopf-Genswein, K. S. *et al.* Relationship between feeding behavior and performance of feedlot steers fed barley-based diets. *J. Anim. Sci.* **89**, 1180-1192, doi:10.2527/jas.2010-3007 (2011).

360 Basarab, J. A., Milligan, D., Hand, R. & Huisman, C. in *Proc. Can. Soc. Anim. Sci. 46th Annu. Conf.* . 28

361 Sowell, B. F. *et al.* Feeding and watering behavior of healthy and morbid steers in a commercial feedlot. *J. Anim. Sci.* **77**, 1105-1112 (1999).

362 Moya, D. *et al.* Use of pattern recognition techniques for early detection of morbidity in receiving feedlot cattle. *J. Anim. Sci.* **93**, 3623-3638 (2015).

363 Jackson, K. S., Carstens, G. E., Tedeschi, L. O. & Pinchak, W. E. Changes in feeding behavior patterns and dry matter intake before clinical symptoms associated with bovine respiratory disease in growing bulls. *J. Anim. Sci.* **94**, 1644-1652, doi:10.2527/jas.2015-9993 (2016).

364 Huzzey, J. M., Veira, D. M., Weary, D. M. & von Keyserlingk, M. A. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. *J. Dairy Sci.* **90**, 3220-3233, doi:10.3168/jds.2006-807 (2007).

365 Gonzalez, L. A., Tolkamp, B. J., Coffey, M. P., Ferret, A. & Kyriazakis, I. Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *J. Dairy Sci.* **91**, 1017-1028, doi:10.3168/jds.2007-0530 (2008).

366 Goldhawk, C., Chapinal, N., Veira, D. M., Weary, D. M. & von Keyserlingk, M. A. Prepartum feeding behavior is an early indicator of subclinical ketosis. *J. Dairy Sci.* **92**, 4971-4977, doi:10.3168/jds.2009-2242 (2009).

367 Costa, J. H., von Keyserlingk, M. A. & Weary, D. M. Invited review: Effects of group housing of dairy calves on behavior, cognition, performance, and health. *J. Dairy Sci.*, doi:10.3168/jds.2015-10144 (2016).

368 Jensen, M. B. & Holm, L. The effect of milk flow rate and milk allowance on feeding related behaviour in dairy calves fed by computer controlled milk feeders. *Appl. Anim. Behav. Sci.* **82**, 87-100, doi:http://dx.doi.org/10.1016/S0168-1591(03)00054-6 (2003).

369 Jensen, M. B. Computer-controlled milk feeding of dairy calves: the effects of number of calves per feeder and number of milk portions on use of feeder and social behavior. *J. Dairy Sci.* **87**, 3428-3438, doi:10.3168/jds.S0022-0302(04)73478-5 (2004).

370 Borderas, T. F., de Passille, A. M. & Rushen, J. Feeding behavior of calves fed small or large amounts of milk. *J. Dairy Sci.* **92**, 2843-2852, doi:10.3168/jds.2008-1886 (2009).

371 Maatje, K., Verhoeff, J., Kremer, W. D., Cruijsen, A. L. & van den Ingh, T. S. Automated feeding of milk replacer and health control of group-housed veal calves. *Vet. Rec.* **133**, 266-270 (1993).

372 Svensson, C. & Jensen, M. B. Short communication: Identification of diseased calves by use of data from automatic milk feeders. *J. Dairy Sci.* **90**, 994-997, doi:10.3168/jds.S0022-0302(07)71584-9 (2007).

373 Borderas, T. F., Rushen, J., von Keyserlingk, M. A. & de Passille, A. M. Automated measurement of changes in feeding behavior of milk-fed calves associated with illness. *J. Dairy Sci.* **92**, 4549-4554, doi:10.3168/jds.2009-2109 (2009).

374 Schirmann, K., Chapinal, N., Weary, D. M., Heuwieser, W. & von Keyserlingk, M. A. G. Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *J. Dairy Sci.* **95**, 3212-3217, doi:http://dx.doi.org/10.3168/jds.2011-4741 (2012).

375 Law, S. E. & Sudweeks, E. M. Electronic transducer for rumination research. *J. Anim. Sci.* **41**, 213-218 (1975).

376 Beauchemin, K. A., Zelin, S., Genner, D. & Buchanan-Smith, J. G. An Automatic System for Quantification of Eating and Ruminating Activities of Dairy Cattle Housed in Stalls. *J. Dairy Sci.* **72**, 2746-2759, doi:10.3168/jds.S0022-0302(89)79418-2.

377 Beauchemin, K. A., Kachanoski, R. G., Schaalje, G. B. & Buchanan-Smith, J. G. Characterizing rumination patterns of dairy cows using spectral analysis. *J. Anim. Sci.* **68**, 3163-3170 (1990).

378 Braun, U., Trosch, L., Nydegger, F. & Hassig, M. Evaluation of eating and rumination behaviour in cows using a noseband pressure sensor. *BMC Vet. Res.* **9**, 164, doi:10.1186/1746-6148-9-164 (2013).

379 Braun, U., Zurcher, S. & Hassig, M. Eating and rumination activity in 10 cows over 10 days. *Res. Vet. Sci.* **101**, 196-198, doi:10.1016/j.rvsc.2015.05.001 (2015).

380 Braun, U., Zurcher, S. & Hassig, M. Evaluation of eating and rumination behaviour in 300 cows of three different breeds using a noseband pressure sensor. *BMC Vet. Res.* **11**, 231, doi:10.1186/s12917-015-0549-8 (2015).

381 Pahl, C., Hartung, E., Grothmann, A., Mahlkow-Nerge, K. & Haeussermann, A. Suitability of feeding and chewing time for estimation of feed intake in dairy cows. *Animal* **10**, 1507-1512, doi:10.1017/s1751731115001366 (2016).

382 Pegorini, V. *et al.* In Vivo Pattern Classification of Ingestive Behavior in Ruminants Using FBG Sensors and Machine Learning. *Sensors (Basel)* **15**, 28456-28471, doi:10.3390/s151128456 (2015).

383 Schirmann, K., von Keyserlingk, M. A., Weary, D. M., Veira, D. M. & Heuwieser, W. Technical note: Validation of a system for monitoring rumination in dairy cows. *J. Dairy Sci.* **92**, 6052-6055, doi:10.3168/jds.2009-2361 (2009).

384 Ambriz-Vilchis, V., Jessop, N. S., Fawcett, R. H., Shaw, D. J. & Macrae, A. I. Comparison of rumination activity measured using rumination collars against direct

visual observations and analysis of video recordings of dairy cows in commercial farm environments. *J. Dairy Sci.* **98**, 1750-1758, doi:10.3168/jds.2014-8565 (2015).

385 Burfeind, O. *et al.* Evaluation of a system for monitoring rumination in heifers and calves. *J. Dairy Sci.* **94**, 426-430, doi:10.3168/jds.2010-3239 (2011).

386 Goldhawk, C., Schwartzkopf-Genswein, K. & Beauchemin, K. A. Technical note: validation of rumination collars for beef cattle. *J. Anim. Sci.* **91**, 2858-2862, doi:10.2527/jas.2012-5908 (2013).

387 Elischer, M. F., Arceo, M. E., Karcher, E. L. & Siegford, J. M. Validating the accuracy of activity and rumination monitor data from dairy cows housed in a pasture-based automatic milking system. *J. Dairy Sci.* **96**, 6412-6422, doi:10.3168/jds.2013-6790 (2013).

388 Borchers, M. R., Chang, Y. M., Tsai, I. C., Wadsworth, B. A. & Bewley, J. M. A validation of technologies monitoring dairy cow feeding, ruminating, and lying behaviors. *J. Dairy Sci.* **99**, 7458-7466, doi:10.3168/jds.2015-10843 (2016).

389 Bikker, J. P. *et al.* Technical note: Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. *J. Dairy Sci.* **97**, 2974-2979, doi:10.3168/jds.2013-7560 (2014).

390 Pahl, C., Hartung, E., Mahlkow-Nerge, K. & Haeussermann, A. Feeding characteristics and rumination time of dairy cows around estrus. *J. Dairy Sci.* **98**, 148-154, doi:10.3168/jds.2014-8025 (2015).

391 Schirmann, K., Chapinal, N., Weary, D. M., Heuwieser, W. & von Keyserlingk, M. A. Short-term effects of regrouping on behavior of prepartum dairy cows. *J. Dairy Sci.* **94**, 2312-2319, doi:10.3168/jds.2010-3639 (2011).

392 Grant, R. J., Colenbrander, V. F. & Albright, J. L. Effect of particle size of forage and rumen cannulation upon chewing activity and laterality in dairy cows. *J. Dairy Sci.* **73**, 3158-3164, doi:10.3168/jds.S0022-0302(90)79005-4 (1990).

393 Byskov, M. V., Nadeau, E., Johansson, B. E. & Norgaard, P. Variations in automatically recorded rumination time as explained by variations in intake of dietary fractions and milk production, and between-cow variation. *J. Dairy Sci.* **98**, 3926-3937, doi:10.3168/jds.2014-8012 (2015).

394 Stone, A. E., Jones, B. W., Becker, C. A. & Bewley, J. M. Influence of breed, milk yield, and temperature-humidity index on dairy cow lying time, neck activity, reticulorumen temperature, and rumination behavior. *J. Dairy Sci.* **100**, 2395-2403, doi:10.3168/jds.2016-11607 (2017).

395 Reith, S. & Hoy, S. Relationship between daily rumination time and estrus of dairy cows. *J. Dairy Sci.* **95**, 6416-6420, doi:10.3168/jds.2012-5316 (2012).

396 Soriani, N., Trevisi, E. & Calamari, L. Relationships between rumination time, metabolic conditions, and health status in dairy cows during the transition period. *J. Anim. Sci.* **90**, 4544-4554, doi:10.2527/jas.2012-5064 (2012).

397 Schirmann, K., Chapinal, N., Weary, D. M., Vickers, L. & von Keyserlingk, M. A. Short communication: Rumination and feeding behavior before and after calving in dairy cows. *J. Dairy Sci.* **96**, 7088-7092, doi:10.3168/jds.2013-7023 (2013).

398 Clark, C. E. *et al.* Rumination and activity levels as predictors of calving for dairy cows. *Animal* **9**, 691-695, doi:10.1017/s1751731114003127 (2015).

399 Buchel, S. & Sundrum, A. Short communication: Decrease in rumination time as an indicator of the onset of calving. *J. Dairy Sci.* **97**, 3120-3127, doi:10.3168/jds.2013-7613 (2014).

400 Pahl, C., Hartung, E., Grothmann, A., Mahlkow-Nerge, K. & Haeussermann, A. Rumination activity of dairy cows in the 24 hours before and after calving. *J. Dairy Sci.* **97**, 6935-6941, doi:10.3168/jds.2014-8194 (2014).

401 Calamari, L. *et al.* Rumination time around calving: an early signal to detect cows at greater risk of disease. *J. Dairy Sci.* **97**, 3635-3647, doi:10.3168/jds.2013-7709 (2014).

402 Kaufman, E. I., LeBlanc, S. J., McBride, B. W., Duffield, T. F. & DeVries, T. J. Association of rumination time with subclinical ketosis in transition dairy cows. *J. Dairy Sci.* **99**, 5604-5618, doi:10.3168/jds.2015-10509 (2016).

403 Stangaferro, M. L., Wijma, R., Caixeta, L. S., Al-Abri, M. A. & Giordano, J. O. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part I. Metabolic and digestive disorders. *J. Dairy Sci.* **99**, 7395-7410, doi:10.3168/jds.2016-10907 (2016).

404 Stangaferro, M. L., Wijma, R., Caixeta, L. S., Al-Abri, M. A. & Giordano, J. O. Use of rumination and activity monitoring for the identification of dairy cows with health

disorders: Part II. Mastitis. *J. Dairy Sci.* **99**, 7411-7421, doi:10.3168/jds.2016-10908 (2016).

405 Stangaferro, M. L., Wijma, R., Caixeta, L. S., Al-Abri, M. A. & Giordano, J. O. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part III. Metritis. *J. Dairy Sci.* **99**, 7422-7433, doi:10.3168/jds.2016-11352 (2016).

406 Steensels, M. *et al.* Towards practical application of sensors for monitoring animal health: the effect of post-calving health problems on rumination duration, activity and milk yield. *J. Dairy Res.* **84**, 132-138, doi:10.1017/s0022029917000176 (2017).

407 Steensels, M. *et al.* Towards practical application of sensors for monitoring animal health; design and validation of a model to detect ketosis. *J. Dairy Res.* **84**, 139-145, doi:10.1017/s0022029917000188 (2017).

408 Nogami, H., Arai, S., Okada, H., Zhan, L. & Itoh, T. Minimized Bolus-Type Wireless Sensor Node with a Built-In Three-Axis Acceleration Meter for Monitoring a Cow's Rumen Conditions. *Sensors (Basel)* **17**, doi:10.3390/s17040687 (2017).

409 Robert, B., White, B. J., Renter, D. G. & Larson, R. L. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. *Comput. Electron. Agric.* **67**, 80-84, doi:http://dx.doi.org/10.1016/j.compag.2009.03.002 (2009).

410 Wolfger, B. *et al.* Technical note: Accuracy of an ear tag-attached accelerometer to monitor rumination and feeding behavior in feedlot cattle. *J. Anim. Sci.* **93**, 3164-3168, doi:10.2527/jas.2014-8802 (2015).

411 White, B. J. *et al.* Clinical, behavioral, and pulmonary changes in calves following inoculation with Mycoplasma bovis. *Am. J. Vet. Res.* **73**, 490-497, doi:10.2460/ajvr.73.4.490 (2012).

412 Theurer, M. E. *et al.* Effect of Mannheimia haemolytica pneumonia on behavior and physiologic responses of calves during high ambient environmental temperatures. *J. Anim. Sci.* **91**, 3917-3929, doi:10.2527/jas.2012-5823 (2013).

413 Guo, Y. *et al.* Using accelerometer, high sample rate GPS and magnetometer data to develop a cattle movement and behaviour model. *Ecol. Modell.* **220**, 2068-2075, doi:http://dx.doi.org/10.1016/j.ecolmodel.2009.04.047 (2009).

414 Robert, B. D., White, B. J., Renter, D. G. & Larson, R. L. Determination of lying behavior patterns in healthy beef cattle by use of wireless accelerometers. *Am. J. Vet. Res.* **72**, 467-473, doi:10.2460/ajvr.72.4.467 (2011).

415 Klefot, J. M. *et al.* Development of a noninvasive system for monitoring dairy cattle sleep. *J. Dairy Sci.* **99**, 8477-8485, doi:10.3168/jds.2015-10695 (2016).

416 Kok, A. *et al.* Technical note: Validation of sensor-recorded lying bouts in lactating dairy cows using a 2-sensor approach. *J. Dairy Sci.* **98**, 7911-7916, doi:10.3168/jds.2015-9554 (2015).

417 Mattachini, G., Riva, E., Bisaglia, C., Pompe, J. C. & Provolo, G. Methodology for quantifying the behavioral activity of dairy cows in freestall barns. *J. Anim. Sci.* **91**, 4899-4907, doi:10.2527/jas.2012-5554 (2013).

418 Ledgerwood, D. N., Winckler, C. & Tucker, C. B. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. *J. Dairy Sci.* **93**, 5129-5139, doi:10.3168/jds.2009-2945 (2010).

419 Ungar, E. D., Nevo, Y., Baram, H. & Arieli, A. Evaluation of the IceTag leg sensor and its derivative models to predict behaviour, using beef cattle on rangeland. *J. Neurosci. Methods*, doi:10.1016/j.jneumeth.2017.06.001 (2017).

420 Trenel, P., Jensen, M. B., Decker, E. L. & Skjoth, F. Technical note: Quantifying and characterizing behavior in dairy calves using the IceTag automatic recording device. *J. Dairy Sci.* **92**, 3397-3401, doi:10.3168/jds.2009-2040 (2009).

421 de Passille, A. M., Jensen, M. B., Chapinal, N. & Rushen, J. Technical note: use of accelerometers to describe gait patterns in dairy calves. *J. Dairy Sci.* **93**, 3287-3293, doi:10.3168/jds.2009-2758 (2010).

422 Bonk, S., Burfeind, O., Suthar, V. S. & Heuwieser, W. Technical note: evaluation of data loggers for measuring lying behavior in dairy calves. *J. Dairy Sci.* **96**, 3265-3271, doi:10.3168/jds.2012-6003 (2013).

423 Swartz, T. H., McGilliard, M. L. & Petersson-Wolfe, C. S. Technical note: The use of an accelerometer for measuring step activity and lying behaviors in dairy calves. *J. Dairy Sci.* **99**, 9109-9113, doi:10.3168/jds.2016-11297 (2016).

424 Lovendahl, P. & Chagunda, M. G. On the use of physical activity monitoring for estrus detection in dairy cows. *J. Dairy Sci.* **93**, 249-259, doi:10.3168/jds.2008-1721 (2010).

425 Aungier, S. P., Roche, J. F., Sheehy, M. & Crowe, M. A. Effects of management and health on the use of activity monitoring for estrus detection in dairy cows. *J. Dairy Sci.* **95**, 2452-2466, doi:10.3168/jds.2011-4653 (2012).

426 Neves, R. C., Leslie, K. E., Walton, J. S. & Leblanc, S. J. Reproductive performance with an automated activity monitoring system versus a synchronized breeding program. *J. Dairy Sci.* **95**, 5683-5693, doi:10.3168/jds.2011-5264 (2012).

427 Chanvallon, A. *et al.* Comparison of three devices for the automated detection of estrus in dairy cows. *Theriogenology* **82**, 734-741, doi:10.1016/j.theriogenology.2014.06.010 (2014).

428 Dolecheck, K. A. *et al.* Behavioral and physiological changes around estrus events identified using multiple automated monitoring technologies. *J. Dairy Sci.* **98**, 8723-8731, doi:10.3168/jds.2015-9645 (2015).

429 Hart, K. D., McBride, B. W., Duffield, T. F. & DeVries, T. J. Effect of frequency of feed delivery on the behavior and productivity of lactating dairy cows. *J. Dairy Sci.* **97**, 1713-1724, doi:10.3168/jds.2013-7504 (2014).

430 Deming, J. A., Bergeron, R., Leslie, K. E. & DeVries, T. J. Associations of housing, management, milking activity, and standing and lying behavior of dairy cows milked in automatic systems. *J. Dairy Sci.* **96**, 344-351, doi:10.3168/jds.2012-5985 (2013).

431 King, M. T., Crossley, R. E. & DeVries, T. J. Impact of timing of feed delivery on the behavior and productivity of dairy cows. *J. Dairy Sci.* **99**, 1471-1482, doi:10.3168/jds.2015-9790 (2016).

432 DeVries, T. J., von Keyserlingk, M. A. & Beauchemin, K. A. Frequency of feed delivery affects the behavior of lactating dairy cows. *J. Dairy Sci.* **88**, 3553-3562, doi:10.3168/jds.S0022-0302(05)73040-X (2005).

433 Devries, T. J. & von Keyserlingk, M. A. Time of feed delivery affects the feeding and lying patterns of dairy cows. *J. Dairy Sci.* **88**, 625-631, doi:10.3168/jds.S0022-0302(05)72726-0 (2005).

434 Miller-Cushon, E. K. & DeVries, T. J. Short communication: Associations between feed push-up frequency, feeding and lying behavior, and milk yield and composition of dairy cows. *J. Dairy Sci.*, doi:10.3168/jds.2016-12004 (2017).

435 DeVries, T. J., Dufour, S. & Scholl, D. T. Relationship between feeding strategy, lying behavior patterns, and incidence of intramammary infection in dairy cows. *J. Dairy Sci.* **93**, 1987-1997, doi:10.3168/jds.2009-2692 (2010).

436 Devries, T. J. *et al.* Association of standing and lying behavior patterns and incidence of intramammary infection in dairy cows milked with an automatic milking system. *J. Dairy Sci.* **94**, 3845-3855, doi:10.3168/jds.2010-4032 (2011).

437 Watters, M. E. *et al.* Associations of herd- and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows. *Prev. Vet. Med.* **111**, 245-255, doi:10.1016/j.prevetmed.2013.05.015 (2013).

438 Thompson, A. J., Weary, D. M. & von Keyserlingk, M. A. G. Technical note: Mining data from on-farm electronic equipment to identify the time dairy cows spend away from the pen. *J. Dairy Sci.* **100**, 3975-3982, doi:10.3168/jds.2016-11713 (2017).

439 Barrier, A. C., Haskell, M. J., Macrae, A. I. & Dwyer, C. M. Parturition progress and behaviours in dairy cows with calving difficulty. *Appl. Anim. Behav. Sci.* **139**, 209-217, doi:http://dx.doi.org/10.1016/j.applanim.2012.03.003 (2012).

440 Jensen, M. B. Behavior around the time of calving in dairy cows. *Appl. Anim. Behav. Sci.* **139**, 195-202 (2012).

441 Miedema, H. M., Cockram, M. S., Dwyer, C. M. & Macrae, A. I. Changes in the behaviour of dairy cows during the 24 h before normal calving compared with behaviour during late pregnancy *Appl. Anim. Behav. Sci.* **131**, 8-14 (2011).

442 Huzzey, J. M., von Keyserlingk, M. A. & Weary, D. M. Changes in feeding, drinking, and standing behavior of dairy cows during the transition period. *J. Dairy Sci.* **88**, 2454-2461, doi:10.3168/jds.S0022-0302(05)72923-4 (2005).

443 Titler, M. *et al.* Prediction of parturition in Holstein dairy cattle using electronic data loggers. *J. Dairy Sci.* **98**, 5304-5312, doi:10.3168/jds.2014-9223 (2015).

444 Borchers, M. R. *et al.* Machine-learning-based calving prediction from activity, lying, and ruminating behaviors in dairy cattle. *J. Dairy Sci.*, doi:10.3168/jds.2016-11526 (2017).

445 Rutten, C. J., Steeneveld, W., Kamphuis, C., Huijps, K. & Hogeveen, H. in *7th European Conference on Precision Livestock Farming* 397-405 (ECPLF).

446 Santegoeds, O. J. *Predicting dairy cow parturition using real-time behavior data from accelerometers* Master of Science thesis, Delft University of Technology (2016).

447 Rutten, C. J. *et al.* Sensor data on cow activity, rumination, and ear temperature improve prediction of the start of calving in dairy cows. *Comput. Electron. Agric.* **132**, 108-118, doi:10.1016/j.compag.2016.11.009 (2017).

448 Rutten, C. J. *et al.* Sensor data on cow activity, rumination, and ear temperature improve prediction of the start of calving in dairy cows. *Comput. Electron. Agric.* **132**, 108-118, doi:http://dx.doi.org/10.1016/j.compag.2016.11.009 (2017).

449 Zimov, J. L., Botheras, N. A., Weiss, W. P. & Hogan, J. S. Associations among behavioral and acute physiologic responses to lipopolysaccharide-induced clinical mastitis in lactating dairy cows. *Am. J. Vet. Res.* **72**, 620-627, doi:10.2460/ajvr.72.5.620 (2011).

450 Siivonen, J. *et al.* Impact of acute clinical mastitis on cow behavior *Appl. Anim. Behav. Sci.* **132**, 101-106 (2011).

451 Fogsgaard, K. K., Rontved, C. M., Sorensen, P. & Herskin, M. S. Sickness behavior in dairy cows during Escherichia coli mastitis. *J. Dairy Sci.* **95**, 630-638, doi:10.3168/jds.2011-4350 (2012).

452 Cyples, J. A. *et al.* Short communication: The effects of experimentally induced Escherichia coli clinical mastitis on lying behavior of dairy cows. *J. Dairy Sci.* **95**, 2571-2575, doi:10.3168/jds.2011-5135 (2012).

453 de Boyer des Roches, A. *et al.* Behavioral and patho-physiological response as possible signs of pain in dairy cows during Escherichia coli mastitis: A pilot study. *J. Dairy Sci.*, doi:10.3168/jds.2017-12796 (2017).

454 Medrano-Galarza, C., Gibbons, J., Wagner, S., de Passille, A. M. & Rushen, J. Behavioral changes in dairy cows with mastitis. *J. Dairy Sci.* **95**, 6994-7002, doi:10.3168/jds.2011-5247 (2012).

455 Fogsgaard, K. K., Bennedsgaard, T. W. & Herskin, M. S. Behavioral changes in freestall-housed dairy cows with naturally occurring clinical mastitis. *J. Dairy Sci.* **98**, 1730-1738, doi:10.3168/jds.2014-8347 (2015).

456 Jawor, P. E., Huzzey, J. M., LeBlanc, S. J. & von Keyserlingk, M. A. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing

behaviors around parturition in Holstein cows. *J. Dairy Sci.* **95**, 1240-1248, doi:10.3168/jds.2011-4586 (2012).

457 Itle, A. J., Huzzey, J. M., Weary, D. M. & von Keyserlingk, M. A. Clinical ketosis and standing behavior in transition cows. *J. Dairy Sci.* **98**, 128-134, doi:10.3168/jds.2014-7932 (2015).

458 Kaufman, E. I., LeBlanc, S. J., McBride, B. W., Duffield, T. F. & DeVries, T. J. Short communication: Association of lying behavior and subclinical ketosis in transition dairy cows. *J. Dairy Sci.* **99**, 7473-7480, doi:10.3168/jds.2016-11185 (2016).

459 Edwards, J. L. & Tozer, P. R. Using activity and milk yield as predictors of fresh cow disorders. *J. Dairy Sci.* **87**, 524-531, doi:10.3168/jds.S0022-0302(04)73192-6 (2004).

460 Liboreiro, D. N. *et al.* Characterization of peripartum rumination and activity of cows diagnosed with metabolic and uterine diseases. *J. Dairy Sci.* **98**, 6812-6827, doi:10.3168/jds.2014-8947 (2015).

461 Sepulveda-Varas, P., Weary, D. M. & von Keyserlingk, M. A. Lying behavior and postpartum health status in grazing dairy cows. *J. Dairy Sci.* **97**, 6334-6343, doi:10.3168/jds.2014-8357 (2014).

462 Hanzlicek, G. A., White, B. J., Mosier, D., Renter, D. G. & Anderson, D. E. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of experimentally induced Mannheimia haemolytica pneumonia in postweaned calves. *Am. J. Vet. Res.* **71**, 359-369, doi:10.2460/ajvr.71.3.359 (2010).

463 Bayne, J. E. *et al.* Use of three-dimensional accelerometers to evaluate behavioral changes in cattle experimentally infected with bovine viral diarrhea virus. *Am. J. Vet. Res.* **77**, 589-596, doi:10.2460/ajvr.77.6.589 (2016).

464 Smith, J. L., Vanzant, E. S., Carter, C. N. & Jackson, C. B. Discrimination of healthy versus sick steers by means of continuous remote monitoring of animal activity. *Am. J. Vet. Res.* **76**, 739-744, doi:10.2460/ajvr.76.8.739 (2015).

465 Cha, E., Hertl, J. A., Bar, D. & Grohn, Y. T. The cost of different types of lameness in dairy cows calculated by dynamic programming. *Prev. Vet. Med.* **97**, 1-8, doi:10.1016/j.prevetmed.2010.07.011 (2010).

466 Liang, D., Arnold, L. M., Stowe, C. J., Harmon, R. J. & Bewley, J. M. Estimating US dairy clinical disease costs with a stochastic simulation model. *J. Dairy Sci.* **100**, 1472-1486, doi:10.3168/jds.2016-11565 (2017).

467 von Keyserlingk, M. A., Barrientos, A., Ito, K., Galo, E. & Weary, D. M. Benchmarking cow comfort on North American freestall dairies: lameness, leg injuries, lying time, facility design, and management for high-producing Holstein dairy cows. *J. Dairy Sci.* **95**, 7399-7408, doi:10.3168/jds.2012-5807 (2012).

468 Terrell, S. P. *et al.* Perception of lameness management, education, and effects on animal welfare of feedlot cattle by consulting nutritionists, veterinarians, and feedlot managers *Bovine Practitioner* **48**, 53-60 (2014).

469 Sprecher, D. J., Hostetler, D. E. & Kaneene, J. B. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology* **47**, 1179-1187 (1997).

470 O'Callaghan, K. A., Cripps, P. J., Downham, D. Y. & Murray, R. D. Subjective and objective assessment of pain and discomfort due to lameness in dairy cattle. *Anim. Welfare* **12**, 605-610 (2003).

471 FARM. (http://nationaldairyfarm.com/sites/default/files/Version%203.0%20References%20and%20Appendices.pdf, 2016).

472 (http://www.zinpro.com/lameness/beef/locomotion-scoring, 2016).

473 NAMI. (https://www.youtube.com/watch?v5QIslfHCvkpg, 2016).

474 Otten, N. D., Toft, N., Houe, H., Thomsen, P. T. & Sorensen, J. T. Adjusting for multiple clinical observers in an unbalanced study design using latent class models of true within-herd lameness prevalence in Danish dairy herds. *Prev. Vet. Med.* **112**, 348-354, doi:10.1016/j.prevetmed.2013.09.002 (2013).

475 Van Nuffel, A., Sprenger, M., Tuyttens, F. & Maertens, W. *Cow gait scores and kinematic gait data: Can people see gait irregularities?* , Vol. 18 (2009).

476 Fuller, C. J., Bladon, B. M., Driver, A. J. & Barr, A. R. The intra- and inter-assessor reliability of measurement of functional outcome by lameness scoring in horses. *Vet. J.* **171**, 281-286, doi:10.1016/j.tvjl.2004.10.012 (2006).

477 Hewetson, M., Christley, R. M., Hunt, I. D. & Voute, L. C. Investigations of the reliability of observational gait analysis for the assessment of lameness in horses. *Vet. Rec.* **158**, 852-857 (2006).

478 Gregory, B. in *The Athletic Horse (SECOND EDITION)* (eds Kenneth Harrington McKeever & Catherine M. McGowan) 266-281 (W.B. Saunders, 2014).

479 Scott, G. B. Changes in limb loading with lameness for a number of Friesian cattle. *Br. Vet. J.* **145**, 28-38, doi:10.1016/0007-1935(89)90005-5 (1989).

480 van der Tol, P. P. *et al.* The vertical ground reaction force and the pressure distribution on the claws of dairy cows while walking on a flat substrate. *J. Dairy Sci.* **86**, 2875-2883, doi:10.3168/jds.S0022-0302(03)73884-3 (2003).

481 Chapinal, N., de Passille, A. M. & Rushen, J. Weight distribution and gait in dairy cattle are affected by milking and late pregnancy. *J. Dairy Sci.* **92**, 581-588, doi:10.3168/jds.2008-1533 (2009).

482 Chapinal, N., de Passille, A. M., Rushen, J. & Tucker, C. B. Short communication: measures of weight distribution and frequency of steps as indicators of restless behavior. *J. Dairy Sci.* **94**, 800-803, doi:10.3168/jds.2010-3531 (2011).

483 Pastell, M. E. & Kujala, M. A probabilistic neural network model for lameness detection. *J. Dairy Sci.* **90**, 2283-2292, doi:10.3168/jds.2006-267 (2007).

484 Chapinal, N. & Tucker, C. B. Validation of an automated method to count steps while cows stand on a weighing platform and its application as a measure to detect lameness. *J. Dairy Sci.* **95**, 6523-6528, doi:10.3168/jds.2012-5742 (2012).

485 Chapinal, N., de Passille, A. M., Rushen, J. & Wagner, S. Automated methods for detecting lameness and measuring analgesia in dairy cattle. *J. Dairy Sci.* **93**, 2007-2013, doi:10.3168/jds.2009-2803 (2010).

486 Liu, J., Dyer, R. M., Neerchal, N. K., Tasch, U. & Rajkondawar, P. G. Diversity in the magnitude of hind limb unloading occurs with similar forms of lameness in dairy cows. *J. Dairy Res.* **78**, 168-177, doi:10.1017/s0022029911000057 (2011).

487 Pastell, M., Hanninen, L., de Passille, A. M. & Rushen, J. Measures of weight distribution of dairy cows to detect lameness and the presence of hoof lesions. *J. Dairy Sci.* **93**, 954-960, doi:10.3168/jds.2009-2385 (2010).

488 Ghotoorlar, S. M., Ghamsari, S. M., Nowrouzian, I., Ghotoorlar, S. M. & Ghidary, S. S. Lameness scoring system for dairy cows using force plates and artificial intelligence. *Vet. Rec.* **170**, 126, doi:10.1136/vr.100429 (2012).

489 Bicalho, R. C., Cheong, S. H., Cramer, G. & Guard, C. L. Association between a visual and an automated locomotion score in lactating Holstein cows. *J. Dairy Sci.* **90**, 3294-3300, doi:10.3168/jds.2007-0076 (2007).

490 Chapinal, N., de Passille, A. M. & Rushen, J. Correlated changes in behavioral indicators of lameness in dairy cows following hoof trimming. *J. Dairy Sci.* **93**, 5758-5763, doi:10.3168/jds.2010-3426 (2010).

491 Chapinal, N., de Passille, A. M., Rushen, J. & Wagner, S. A. Effect of analgesia during hoof trimming on gait, weight distribution, and activity of dairy cattle. *J. Dairy Sci.* **93**, 3039-3046, doi:10.3168/jds.2009-2987 (2010).

492 Dunthorn, J. *et al.* Predictive models of lameness in dairy cows achieve high sensitivity and specificity with force measurements in three dimensions. *J. Dairy Res.* **82**, 391-399, doi:10.1017/s002202991500028x (2015).

493 Thorup, V. M. *et al.* Short communication: Changes in gait symmetry in healthy and lame dairy cows based on 3-dimensional ground reaction force curves following claw trimming. *J. Dairy Sci.* **97**, 7679-7684, doi:10.3168/jds.2014-8410 (2014).

494 Maertens, W. *et al.* Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: the GAITWISE system *Biosys. Eng.* **110**, 29-39 (2011).

495 Van Nuffel, A. *et al.* Environmental and cow-related factors affect cow locomotion and can cause misclassification in lameness detection systems. *Animal* **10**, 1533-1541, doi:10.1017/s175173111500244x (2016).

496 Wheeler, C. A., White, B. J., Anderson, D. E., Amrine, D. E. & Larson, R. L. Assessment of biometric tools for quantitative gait analysis in Holstein calves. *Am. J. Vet. Res.* **74**, 1443-1449, doi:10.2460/ajvr.74.11.1443 (2013).

497 Kotschwar, J. L. *et al.* Analgesic efficacy of sodium salicylate in an amphotericin B-induced bovine synovitis-arthritis model. *J. Dairy Sci.* **92**, 3731-3743, doi:10.3168/jds.2009-2058 (2009).

498 Schulz, K. L., Anderson, D. E., Coetzee, J. F., White, B. J. & Miesner, M. D. Effect of flunixin meglumine on the amelioration of lameness in dairy steers with amphotericin B-induced transient synovitis-arthritis. *Am. J. Vet. Res.* **72**, 1431-1438, doi:10.2460/ajvr.72.11.1431 (2011).

499 Coetzee, J. F. *et al.* Impact of oral meloxicam administered alone or in combination with gabapentin on experimentally induced lameness in beef calves. *J. Anim. Sci.* **92**, 816-829, doi:10.2527/jas.2013-6999 (2014).

500 Herlin, A. H. & Drevemo, S. Investigating locomotion of dairy cows by use of high speed cinematography. *Equine Vet. J.* **29**, 106-109, doi:10.1111/j.2042-3306.1997.tb05066.x (1997).

501 Flower, F. C., Sanderson, D. J. & Weary, D. M. Hoof pathologies influence kinematic measures of dairy cow gait. *J. Dairy Sci.* **88**, 3166-3173, doi:10.3168/jds.S0022-0302(05)73000-9 (2005).

502 Flower, F. C., Sanderson, D. J. & Weary, D. M. Effects of milking on dairy cow gait. *J. Dairy Sci.* **89**, 2084-2089, doi:10.3168/jds.S0022-0302(06)72278-0 (2006).

503 Flower, F. C., de Passille, A. M., Weary, D. M., Sanderson, D. J. & Rushen, J. Softer, higher-friction flooring improves gait of cows with and without sole ulcers. *J. Dairy Sci.* **90**, 1235-1242, doi:10.3168/jds.S0022-0302(07)71612-0 (2007).

504 Blackie, N., Bleach, E. C., Amory, J. R. & Scaife, J. R. Associations between locomotion score and kinematic measures in dairy cows with varying hoof lesion types. *J. Dairy Sci.* **96**, 3564-3572, doi:10.3168/jds.2012-5597 (2013).

505 Meyer, S. W., Weishaupt, M. A. & Nuss, K. A. Gait pattern of heifers before and after claw trimming: a high-speed cinematographic study on a treadmill. *J. Dairy Sci.* **90**, 670-676, doi:10.3168/jds.S0022-0302(07)71549-7 (2007).

506 Schmid, T. *et al.* High-speed cinematographic evaluation of claw-ground contact pattern of lactating cows. *Vet. J.* **181**, 151-157, doi:10.1016/j.tvjl.2008.02.019 (2009).

507 Chapinal, N. *et al.* Measurement of acceleration while walking as an automated method for gait assessment in dairy cattle. *J. Dairy Sci.* **94**, 2895-2901, doi:10.3168/jds.2010-3882 (2011).

508 Tanida, H., Koba, Y., Rushen, J. & De Passile, A. M. Use of three-dimensional acceleration sensing to assess dairy cow gait and the effects of hoof trimming. *Anim Sci J* **82**, 792-800, doi:10.1111/j.1740-0929.2011.00903.x (2011).

509 Alsaaod, M. *et al.* Development and validation of a novel pedometer algorithm to quantify extended characteristics of the locomotor behavior of dairy cows. *J. Dairy Sci.* **98**, 6236-6242, doi:10.3168/jds.2015-9657 (2015).

510 Alsaaod, M., Luternauer, M., Hausegger, T., Kredel, R. & Steiner, A. The cow pedogram-Analysis of gait cycle variables allows the detection of lameness and foot pathologies. *J. Dairy Sci.* **100**, 1417-1426, doi:10.3168/jds.2016-11678 (2017).

511 Alsaaod, M., Kredel, R., Hofer, B. & Steiner, A. Technical note: Validation of a semi-automated software tool to determine gait-cycle variables in dairy cows. *J. Dairy Sci.* **100**, 4897-4902, doi:10.3168/jds.2016-12235 (2017).

512 Poursaberi, A., Bahr, C., Pluk, A., Van Nuffel, A. & Berckmans, D. Real-time automatic lameness detection based on back posture extraction in dairy cattle: Shape analysis of cow with image processing techniques. *Comput. Electron. Agric.* **74**, 110-119, doi:http://dx.doi.org/10.1016/j.compag.2010.07.004 (2010).

513 Viazzi, S. *et al.* Analysis of individual classification of lameness using automatic measurement of back posture in dairy cattle. *J. Dairy Sci.* **96**, 257-266, doi:http://dx.doi.org/10.3168/jds.2012-5806 (2013).

514 Viazzi, S. *et al.* Comparison of a three-dimensional and two-dimensional camera system for automated measurement of back posture in dairy cows. *Comput. Electron. Agric.* **100**, 139-147, doi:http://dx.doi.org/10.1016/j.compag.2013.11.005 (2014).

515 Van Hertem, T. *et al.* Automatic lameness detection based on consecutive 3D-video recordings. *Biosys. Eng.* **119**, 108-116, doi:http://dx.doi.org/10.1016/j.biosystemseng.2014.01.009 (2014).

516 Mazrier, H., Tal, S., Aizinbud, E. & Bargai, U. A field investigation of the use of the pedometer for the early detection of lameness in cattle. *Can. Vet. J.* **47**, 883-886 (2006).

517 Thorup, V. M. *et al.* Lameness detection via leg-mounted accelerometers on dairy cows on four commercial farms. *Animal* **9**, 1704-1712, doi:10.1017/s1751731115000890 (2015).

518 Alsaaod, M. *et al.* Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. *Appl. Anim. Behav. Sci.* **142**, 134-141, doi:10.1016/j.applanim.2012.10.001.

519 Yunta, C., Guasch, I. & Bach, A. Short communication: Lying behavior of lactating dairy cows is influenced by lameness especially around feeding time. *J. Dairy Sci.* **95**, 6546-6549, doi:10.3168/jds.2012-5670.

520 Westin, R. *et al.* Lying times of lactating cows on dairy farms with automatic milking systems and the relation to lameness, leg lesions, and body condition score. *J. Dairy Sci.* **99**, 551-561, doi:10.3168/jds.2015-9737 (2016).

521 de Mol, R. M. *et al.* Applicability of day-to-day variation in behavior for the automated detection of lameness in dairy cows. *J. Dairy Sci.* **96**, 3703-3712, doi:http://dx.doi.org/10.3168/jds.2012-6305 (2013).

522 Chapinal, N., de Passille, A. M., Weary, D. M., von Keyserlingk, M. A. & Rushen, J. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. *J. Dairy Sci.* **92**, 4365-4374, doi:10.3168/jds.2009-2115 (2009).

523 Nechanitzky, K. *et al.* Analysis of behavioral changes in dairy cows associated with claw horn lesions. *J. Dairy Sci.* **99**, 2904-2914, doi:10.3168/jds.2015-10109 (2016).

524 Van Hertem, T. *et al.* Lameness detection based on multivariate continuous sensing of milk yield, rumination, and neck activity. *J. Dairy Sci.* **96**, 4286-4298, doi:10.3168/jds.2012-6188 (2013).

525 Beer, G. *et al.* Use of Extended Characteristics of Locomotion and Feeding Behavior for Automated Identification of Lame Dairy Cows. *PLoS One* **11**, e0155796, doi:10.1371/journal.pone.0155796 (2016).

526 Takahashi, E., Kuwayama, H., Kawamoto, K., Matsui, T. & Inokuma, H. Detection of serum amyloid A isoforms in cattle. *J. Vet. Diagn. Invest.* **21**, 874-877, doi:10.1177/104063870902100620 (2009).

527 Horadagoda, N. U. *et al.* Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet. Rec.* **144**, 437-441 (1999).

528 Tothova, C., Nagy, O., Seide, H. & Kovac, G. The effect of chronic respiratory diseases on acute phase proteins and selected blood parameters of protein metabolism in calves. *Berl. Munch. Tierarztl. Wochenschr.* **123**, 307-313 (2010).

529 Akerstedt, M., Forsback, L., Larsen, T. & Svennersten-Sjaunja, K. Natural variation in biomarkers indicating mastitis in healthy cows. *J. Dairy Res.* **78**, 88-96, doi:10.1017/s0022029910000786 (2011).

530 Thomas, F. C. *et al.* The major acute phase proteins of bovine milk in a commercial dairy herd. *BMC Vet. Res.* **11**, 207, doi:10.1186/s12917-015-0533-3 (2015).

531 Alsemgeest, S. P. *et al.* Influence of physical stress on the plasma concentration of serum amyloid-A (SAA) and haptoglobin (Hp) in calves. *Vet. Q.* **17**, 9-12, doi:10.1080/01652176.1995.9694565 (1995).

532 Gerardi, G. *et al.* Use of serum amyloid A and milk amyloid A in the diagnosis of subclinical mastitis in dairy cows. *J. Dairy Res.* **76**, 411-417, doi:10.1017/s0022029909990057 (2009).

533 Alsemgeest, S. P. *et al.* 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 (1994).

534 Humblet, M. F. *et al.* Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. *Vet. Clin. Pathol.* **35**, 188-193 (2006).

535 Cooke, R. F., Guarnieri Filho, T. A., Cappellozza, B. I. & Bohnert, D. W. Rest stops during road transport: impacts on performance and acute-phase protein responses of feeder cattle. *J. Anim. Sci.* **91**, 5448-5454, doi:10.2527/jas.2013-6357 (2013).

536 Marques, R. S., Cooke, R. F., Francisco, C. L. & Bohnert, D. W. Effects of twenty-four hour transport or twenty-four hour feed and water deprivation on physiologic and performance responses of feeder cattle. *J. Anim. Sci.* **90**, 5040-5046, doi:10.2527/jas.2012-5425 (2012).

537 Lomborg, S. R., Nielsen, L. R., Heegaard, P. M. & Jacobsen, S. Acute phase proteins in cattle after exposure to complex stress. *Vet. Res. Commun.* **32**, 575-582, doi:10.1007/s11259-008-9057-7 (2008).

538 Kim, M. H. *et al.* The stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in Holstein calves. *J. Vet. Sci.* **12**, 151-157 (2011).

539 Lynch, E. M., Earley, B., McGee, M. & Doyle, S. Effect of abrupt weaning at housing on leukocyte distribution, functional activity of neutrophils, and acute phase protein response of beef calves. *BMC Vet. Res.* **6**, 39, doi:10.1186/1746-6148-6-39 (2010).

540 Lynch, E. M., Earley, B., McGee, M. & Doyle, S. Characterisation of physiological and immunological responses in beef cows to abrupt weaning and subsequent housing. *BMC Vet. Res.* **6**, 37, doi:10.1186/1746-6148-6-37 (2010).

541 Lynch, E. M., McGee, M., Doyle, S. & Earley, B. Effect of pre-weaning concentrate supplementation on peripheral distribution of leukocytes, functional activity of neutrophils, acute phase protein and behavioural responses of abruptly weaned and housed beef calves. *BMC Vet. Res.* **8**, 1, doi:10.1186/1746-6148-8-1 (2012).

542 Warnock, T. M. *et al.* Effect of castration technique on beef calf performance, feed efficiency, and inflammatory response. *J. Anim. Sci.* **90**, 2345-2352, doi:10.2527/jas.2011-4511 (2012).

543 Borghetti, P., Saleri, R., Mocchegiani, E., Corradi, A. & Martelli, P. Infection, immunity and the neuroendocrine response. *Vet. Immunol. Immunopathol.* **130**, 141-162, doi:10.1016/j.vetimm.2009.01.013 (2009).

544 Carroll, J. A. *et al.* Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. *Innate Immun.* **15**, 81-89, doi:10.1177/1753425908099170 (2009).

545 Plessers, E. *et al.* Characterization of an intravenous lipopolysaccharide inflammation model in calves with respect to the acute-phase response. *Vet. Immunol. Immunopathol.* **163**, 46-56, doi:10.1016/j.vetimm.2014.11.005 (2015).

546 Dantzer, R. Cytokine-induced sickness behavior: mechanisms and implications. *Ann. N. Y. Acad. Sci.* **933**, 222-234 (2001).

547 McMahon, C. D. *et al.* Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. *J. Endocrinol.* **161**, 333-339 (1999).

548 Passler, T. *et al.* Evaluation of methods to improve the diagnosis of systemic inflammation in alpacas. *J. Vet. Intern. Med.* **27**, 970-976, doi:10.1111/jvim.12102 (2013).

549 Faul, F., Erdfelder, E., Lang, A. G. & Buchner, A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* **39**, 175-191 (2007).

550 Borderas, T. F., de Passille, A. M. & Rushen, J. Behavior of dairy calves after a low dose of bacterial endotoxin. *J. Anim. Sci.* **86**, 2920-2927, doi:10.2527/jas.2008-0926 (2008).

551 Steiger, M. *et al.* Effect of a prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. *J. Anim. Sci.* **77**, 2523-2532 (1999).

552 Werling, D. *et al.* Characterisation of the acute phase response of heifers to a prolonged low dose infusion of lipopolysaccharide. *Res. Vet. Sci.* **61**, 252-257 (1996).

553 Jacobsen, S., Andersen, P. H., Toelboell, T. & Heegaard, P. M. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. *J. Dairy Sci.* **87**, 3330-3339, doi:10.3168/jds.S0022-0302(04)73469-4 (2004).

554 O'Reilly, B., Vander, A. J. & Kluger, M. J. Effects of chronic infusion of lipopolysaccharide on food intake and body temperature of the rat. *Physiol. Behav.* **42**, 287-291 (1988).

555 Goran, M. I., Frayn, K. N. & Little, R. A. Energy metabolism during chronic endotoxin infusion in the rat, with special reference to free fatty acid turnover. *Circ. Shock* **25**, 9-20 (1988).

556 Goran, M. I., Little, R. A., Frayn, K. N., Jones, R. & Fozzard, G. Effects of chronic endotoxaemia on oxygen consumption at different ambient temperatures in the unanaesthetised rat. *Circ. Shock* **25**, 103-109 (1988).

557 Ayala, A., Deol, Z. K., Lehman, D. L., Herdon, C. D. & Chaudry, I. H. Polymicrobial sepsis but not low-dose endotoxin infusion causes decreased splenocyte IL-2/IFN-gamma release while increasing IL-4/IL-10 production. *J. Surg. Res.* **56**, 579-585, doi:10.1006/jsre.1994.1092 (1994).

558 Cani, P. D. *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761-1772, doi:10.2337/db06-1491 (2007).

559 Nguyen, A. T. *et al.* Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: involvement of the GLP-1 pathway. *Diabetes* **63**, 471-482, doi:10.2337/db13-0903 (2014).

560 Waggoner, J. W., Loest, C. A., Turner, J. L., Mathis, C. P. & Hallford, D. M. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. *J. Anim. Sci.* **87**, 3656-3668, doi:10.2527/jas.2009-2011 (2009).

561 Hessman, B. E. *et al.* Evaluation of economic effects and the health and performance of the general cattle population after exposure to cattle persistently infected with bovine viral diarrhea virus in a starter feedlot. *Am. J. Vet. Res.* **70**, 73-85, doi:10.2460/ajvr.70.1.73 (2009).

562 Jericho, K. W. & Kozub, G. C. Experimental infectious respiratory disease in groups of calves: lobar distribution, variance, and sample-size requirements for vaccine evaluation. *Can. J. Vet. Res.* **68**, 118-127 (2004).

563 Archambault, D., Beliveau, C., Couture, Y. & Carman, S. Clinical response and immunomodulation following experimental challenge of calves with type 2 noncytopathogenic bovine viral diarrhea virus. *Vet. Res.* **31**, 215-227, doi:10.1051/vetres:2000117 (2000).

564 Al-Haddawi, M., Mitchell, G. B., Clark, M. E., Wood, R. D. & Caswell, J. L. Impairment of innate immune responses of airway epithelium by infection with bovine viral diarrhea virus. *Vet. Immunol. Immunopathol.* **116**, 153-162, doi:10.1016/j.vetimm.2007.01.007 (2007).

565 Silflow, R. M., Degel, P. M. & Harmsen, A. G. Bronchoalveolar immune defense in cattle exposed to primary and secondary challenge with bovine viral diarrhea virus. *Vet. Immunol. Immunopathol.* **103**, 129-139, doi:10.1016/j.vetimm.2004.09.008 (2005).

566 Walz, P. H. *et al.* Relationship between degree of viremia and disease manifestation in calves with experimentally induced bovine viral diarrhea virus infection. *Am. J. Vet. Res.* **62**, 1095-1103 (2001).

567 Loneragan, G. H., Thomson, D. U., Montgomery, D. L., Mason, G. L. & Larson, R. L. Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. *J. Am. Vet. Med. Assoc.* **226**, 595-601 (2005).

568 Walz, P. H. *et al.* Control of bovine viral diarrhea virus in ruminants. *J. Vet. Intern. Med.* **24**, 476-486, doi:10.1111/j.1939-1676.2010.0502.x (2010).

569 Ellis, J. A. *et al.* Lesions and distribution of viral antigen following an experimental infection of young seronegative calves with virulent bovine virus diarrhea virus-type II. *Can. J. Vet. Res.* **62**, 161-169 (1998).

570 Endres, M. I. & Barberg, A. E. Behavior of dairy cows in an alternative bedded-pack housing system. *J. Dairy Sci.* **90**, 4192-4200, doi:10.3168/jds.2006-751 (2007).

571 Reed, L. J. & Muench, H. A SIMPLE METHOD OF ESTIMATING FIFTY PER CENT ENDPOINTS12. *Am. J. Epidemiol.* **27**, 493-497, doi:10.1093/oxfordjournals.aje.a118408 (1938).

572 Reeve-Johnson, L. Relationships between clinical and pathological signs of disease in calves infected with Mannheimia (Pasteurella) haemolytica type A1. *Vet. Rec.* **149**, 549-552 (2001).

573 Dargatz, D. A., Dewell, G. A. & Mortimer, R. G. Calving and calving management of beef cows and heifers on cow-calf operations in the United States. *Theriogenology* **61**, 997-1007, doi:10.1016/s0093-691x(03)00145-6 (2004).

574 Patterson, D. J., Bellows, R. A., Burfening, P. J. & Carr, J. B. Occurrence of neonatal and postnatal mortality in range beef cattle. I. Calf loss incidence from birth to weaning, backward and breech presentations and effects of calf loss on subsequent pregnancy rate of dams. *Theriogenology* **28**, 557-571 (1987).

575 Wittum, T. E., Salman, M. D., Odde, K. G., Mortimer, R. G. & King, M. E. Causes and costs of calf mortality in Colorado beef herds participating in the National Animal Health Monitoring System. *J. Am. Vet. Med. Assoc.* **203**, 232-236 (1993).

576 Nix, J. M., Spitzer, J. C., Grimes, L. W., Burns, G. L. & Plyler, B. B. A retrospective analysis of factors contributing to calf mortality and dystocia in beef cattle. *Theriogenology* **49**, 1515-1523 (1998).

577 Waldner, C. L., Kennedy, R. I., Rosengren, L. B., Pollock, C. M. & Clark, E. T. Gross postmortem and histologic examination findings from abortion losses and calf mortalities in western Canadian beef herds. *Can. Vet. J.* **51**, 1227-1238 (2010).

578 Holland, M. D. *et al.* Factors contributing to dystocia due to fetal malpresentation in beef cattle. *Theriogenology* **39**, 899-908 (1993).

579 Waldner, C. L. Cow attributes, herd management, and reproductive history events associated with abortion in cow-calf herds from Western Canada. *Theriogenology* **81**, 840-848, doi:10.1016/j.theriogenology.2013.12.016 (2014).

580 (ed CEAH. USDA-APHIS-VS) (Fort Collins, CO 2007-08).

581 Waldner, C. L. & Garcia Guerra, A. Cow attributes, herd management, and reproductive history events associated with the risk of nonpregnancy in cow-calf herds in Western Canada. *Theriogenology* **79**, 1083-1094, doi:10.1016/j.theriogenology.2013.02.004 (2013).

582 Rogers, P. L., Gaskins, C. T., Johnson, K. A. & MacNeil, M. D. Evaluating longevity of composite beef females using survival analysis techniques. *J. Anim. Sci.* **82**, 860-866 (2004).

583 Szabó, F. & Dákay, I. Estimation of some productive and reproductive effects on longevity of beef cows using survival analysis. *Livestock Science* **122**, 271-275, doi:http://dx.doi.org/10.1016/j.livsci.2008.09.024 (2009).

584 Lidfors, L. & Jensen, P. Behaviour of free-ranging beef cows and calves. *Appl. Anim. Behav. Sci.* **20**, 237-247 (1988).

585 Proudfoot, K. L., Jensen, M. B., Weary, D. M. & von Keyserlingk, M. A. Dairy cows seek isolation at calving and when ill. *J. Dairy Sci.* **97**, 2731-2739, doi:10.3168/jds.2013-7274 (2014).

586 Proudfoot, K. L., Weary, D. M. & von Keyserlingk, M. A. Maternal isolation behavior of Holstein dairy cows kept indoors. *J. Anim. Sci.* **92**, 277-281, doi:10.2527/jas.2013-6648 (2014).

587 Wehrend, A., Hofmann, E., Failing, K. & Bostedt, H. Behaviour during the first stage of labour in cattle: Influence of parity and dystocia. *Appl. Anim. Behav. Sci.* **100**, 164-170, doi:http://dx.doi.org/10.1016/j.applanim.2005.11.008 (2006).

588 Dufty, J. H. Determination of the onset of parturition in hereford cattle. *Aust. Vet. J.* **47**, 77-82 (1971).

589 Miedema, H. M., Cockram, M. S., Dwyer, C. M. & Macrae, A. I. Behavioural predictors of the start of normal and dystocic calving in dairy cows and heifers. *Appl. Anim. Behav. Sci.* **132**, 14-19 (2011).

590 in *Current Therapy in Large Animal Theriogenology (Second Edition)* (ed Walter R. Threlfall) iv (W.B. Saunders, 2007).

591 Shah, K. D., Nakao, T. & Kubota, H. Plasma estrone sulphate (E1S) and estradiol-17beta (E2beta) profiles during pregnancy and their relationship with the relaxation of sacrosciatic ligament, and prediction of calving time in Holstein-Friesian cattle. *Anim. Reprod. Sci.* **95**, 38-53, doi:10.1016/j.anireprosci.2005.09.003 (2006).

592 Matsas, D. J., Nebel, R. L. & Pelzer, K. D. Evaluation of an on-farm blood progesterone test for predicting the day of parturition in cattle. *Theriogenology* **37**, 859-868 (1992).

593 Streyl, D. *et al.* Establishment of a standard operating procedure for predicting the time of calving in cattle. *J. Vet. Sci.* **12**, 177-185 (2011).

594 Burfeind, O., Suthar, V. S., Voigtsberger, R., Bonk, S. & Heuwieser, W. Validity of prepartum changes in vaginal and rectal temperature to predict calving in dairy cows. *J. Dairy Sci.* **94**, 5053-5061, doi:10.3168/jds.2011-4484 (2011).

595 Braun, U., Tschoner, T. & Hassig, M. Evaluation of eating and rumination behaviour using a noseband pressure sensor in cows during the peripartum period. *BMC Vet. Res.* **10**, 195, doi:10.1186/s12917-014-0195-6 (2014).

596 Proudfoot, K. L., Huzzey, J. M. & von Keyserlingk, M. A. The effect of dystocia on the dry matter intake and behavior of Holstein cows. *J. Dairy Sci.* **92**, 4937-4944, doi:10.3168/jds.2009-2135 (2009).

597 Bueno, L., Tainturier, D. & Ruckebusch, Y. Detection of parturition in cow and mare by a useful warning system. *Theriogenology* **16**, 599-605, doi:http://dx.doi.org/10.1016/0093-691X(81)90022-4 (1981).

598 Kimura, E., Fujimoto, Y., Sawada, T., Matsunaga, H. & Mori, J. Frequency of Tail Movements before Parturition in Holstein Cows. *Nihon Chikusan Gakkaiho* **63**, 162-166, doi:10.2508/chikusan.63.162 (1992).

599 Cangar, Ö. *et al.* Automatic real-time monitoring of locomotion and posture behaviour of pregnant cows prior to calving using online image analysis. *Comput. Electron. Agric.* **64**, 53-60, doi:http://dx.doi.org/10.1016/j.compag.2008.05.014 (2008).

600 Lombard, J. E., Garry, F. B., Tomlinson, S. M. & Garber, L. P. Impacts of dystocia on health and survival of dairy calves. *J. Dairy Sci.* **90**, 1751-1760, doi:10.3168/jds.2006-295 (2007).

601 Szenci, O. Effects of type and intensity of assistance on acid-base balance of newborn calves. *Acta Vet. Hung.* **31**, 73-79 (1983).

602 Boyd, J. W. Relationships between acid-base balance, serum composition and colostrum absorption in newborn calves. *Br. Vet. J.* **145**, 249-256, doi:10.1016/0007-1935(89)90077-8 (1989).

603 Besser, T. E., Szenci, O. & Gay, C. C. Decreased colostral immunoglobulin absorption in calves with postnatal respiratory acidosis. *J. Am. Vet. Med. Assoc.* **196**, 1239-1243 (1990).

604 Weaver, D. M., Tyler, J. W., VanMetre, D. C., Hostetler, D. E. & Barrington, G. M. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* **14**, 569-577 (2000).

605 Olson, D. P., Papasian, C. J. & Ritter, R. C. The effects of cold stress on neonatal calves. II. Absorption of colostral immunoglobulins. *Can. J. Comp. Med.* **44**, 19-23 (1980).

606 Olson, D. P., Papasian, C. J. & Ritter, R. C. The effects of cold stress on neonatal calves. I. Clinical condition and pathological lesions. *Can. J. Comp. Med.* **44**, 11-18 (1980).

607 Furman-Fratczak, K., Rzasa, A. & Stefaniak, T. The influence of colostral immunoglobulin concentration in heifer calves' serum on their health and growth. *J. Dairy Sci.* **94**, 5536-5543, doi:10.3168/jds.2010-3253 (2011).

608 DeNise, S. K., Robison, J. D., Stott, G. H. & Armstrong, D. V. Effects of passive immunity on subsequent production in dairy heifers. *J. Dairy Sci.* **72**, 552-554, doi:10.3168/jds.S0022-0302(89)79140-2 (1989).

609 Dewell, R. D. *et al.* Association of neonatal serum immunoglobulin G1 concentration with health and performance in beef calves. *J. Am. Vet. Med. Assoc.* **228**, 914-921, doi:10.2460/javma.228.6.914 (2006).

610 Chigerwe, M., Hagey, J. V. & Aly, S. S. Determination of neonatal serum immunoglobulin G concentrations associated with mortality during the first 4 months of life in dairy heifer calves. *J. Dairy Res.* **82**, 400-406, doi:10.1017/s0022029915000503 (2015).

611 Chigerwe, M. *et al.* Evaluation of factors affecting serum IgG concentrations in bottle-fed calves. *J. Am. Vet. Med. Assoc.* **234**, 785-789, doi:10.2460/javma.234.6.785 (2009).

612 Windeyer, M. C. *et al.* Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Prev. Vet. Med.* **113**, 231-240, doi:10.1016/j.prevetmed.2013.10.019 (2014).

613 Wittum, T. E. & Perino, L. J. Passive immune status at postpartum hour 24 and long-term health and performance of calves. *Am. J. Vet. Res.* **56**, 1149-1154 (1995).

614 Tyler, J. W., Hancock, D. D., Thorne, J. G., Gay, C. C. & Gay, J. M. Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. *J. Vet. Intern. Med.* **13**, 335-337 (1999).

615 Virtala, A. M., Grohn, Y. T., Mechor, G. D. & Erb, H. N. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. *Prev. Vet. Med.* **39**, 25-37 (1999).

616 Filteau, V., Bouchard, E., Fecteau, G., Dutil, L. & DuTremblay, D. Health status and risk factors associated with failure of passive transfer of immunity in newborn beef calves in Quebec. *Can. Vet. J.* **44**, 907-913 (2003).

617 Beam, A. L. *et al.* Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. *J. Dairy Sci.* **92**, 3973-3980, doi:10.3168/jds.2009-2225 (2009).

618 (ed USDA APHIS) (National Health Animal Monitoring System Fort Collins, CO 1992).

619 Godden, S. Colostrum management for dairy calves. *Vet. Clin. North Am. Food Anim. Pract.* **24**, 19-39, doi:10.1016/j.cvfa.2007.10.005 (2008).

620 Tyler, J. W. *et al.* Evaluation of 3 assays for failure of passive transfer in calves. *J. Vet. Intern. Med.* **10**, 304-307 (1996).

621 Baxter, E. M. *et al.* Investigating the behavioural and physiological indicators of neonatal survival in pigs. *Theriogenology* **69**, 773-783, doi:10.1016/j.theriogenology.2007.12.007 (2008).

622 Dwyer, C. M. Maternal behaviour and lamb survival: from neuroendocrinology to practical application. *Animal* **8**, 102-112, doi:10.1017/s1751731113001614 (2014).

623 Dwyer, C. M. Behavioural development in the neonatal lamb: effect of maternal and birth-related factors. *Theriogenology* **59**, 1027-1050 (2003).

624 Dwyer, C. M. & Bunger, L. Factors affecting dystocia and offspring vigour in different sheep genotypes. *Prev. Vet. Med.* **103**, 257-264, doi:10.1016/j.prevetmed.2011.09.002 (2012).

625 Dwyer, C. M., Dingwall, W. S. & Lawrence, A. B. Physiological correlates of maternal-offspring behaviour in sheep: a factor analysis. *Physiol. Behav.* **67**, 443-454 (1999).

626 Schuijt, G. & Taverne, M. A. The interval between birth and sternal recumbency as an objective measure of the vitality of newborn calves. *Vet. Rec.* **135**, 111-115 (1994).

627 Schulz, J., Plischke, B. & Braun, H. [Sucking and drinking behavior as criteria of vitality in newborn calves]. *Tierarztl. Prax.* **25**, 116-122 (1997).

628 Murray, C. F. & Leslie, K. E. Newborn calf vitality: risk factors, characteristics, assessment, resulting outcomes and strategies for improvement. *Vet. J.* **198**, 322-328, doi:10.1016/j.tvjl.2013.06.007 (2013).

629 Taverne, M. A. The relation between the birth process and the condition of the newborn piglet and calf. *Vet. Res. Commun.* **32 Suppl 1**, S93-98, doi:10.1007/s11259-008-9094-2 (2008).

630 Mee, J. in *41st Annual Conference Proceedings of the American Association of Bovine Practitioners* 46-53.

631 Cramer, M. C. & Stanton, A. L. Associations between health status and the probability of approaching a novel object or stationary human in preweaned group-housed dairy calves. *J. Dairy Sci.* **98**, 7298-7308, doi:10.3168/jds.2015-9534 (2015).

632 De Paula Vieira, A., de Passille, A. M. & Weary, D. M. Effects of the early social environment on behavioral responses of dairy calves to novel events. *J. Dairy Sci.* **95**, 5149-5155, doi:10.3168/jds.2011-5073 (2012).

633 De Paula Vieira, A., von Keyserlingk, M. A. & Weary, D. M. Presence of an older weaned companion influences feeding behavior and improves performance of dairy calves before and after weaning from milk. *J. Dairy Sci.* **95**, 3218-3224, doi:10.3168/jds.2011-4821 (2012).

634 Duve, L. R. & Jensen, M. B. Social behavior of young dairy calves housed with limited or full social contact with a peer. *J. Dairy Sci.* **95**, 5936-5945, doi:10.3168/jds.2012-5428 (2012).

635 Gaillard, C., Meagher, R. K., von Keyserlingk, M. A. & Weary, D. M. Social housing improves dairy calves' performance in two cognitive tests. *PLoS One* **9**, e90205, doi:10.1371/journal.pone.0090205 (2014).

636 Miguel-Pacheco, G. G., Vaughan, A., de Passille, A. M. & Rushen, J. Relationship between locomotor play of dairy calves and their weight gains and energy intakes around weaning. *Animal* **9**, 1038-1044, doi:10.1017/s1751731115000063 (2015).

637 Costa, J. H., Meagher, R. K., von Keyserlingk, M. A. & Weary, D. M. Early pair housing increases solid feed intake and weight gains in dairy calves. *J. Dairy Sci.* **98**, 6381-6386, doi:10.3168/jds.2015-9395 (2015).

638 De Paula Vieira, A., von Keyserlingk, M. A. & Weary, D. M. Effects of pair versus single housing on performance and behavior of dairy calves before and after weaning from milk. *J. Dairy Sci.* **93**, 3079-3085, doi:10.3168/jds.2009-2516 (2010).

639 Duve, L. R., Weary, D. M., Halekoh, U. & Jensen, M. B. The effects of social contact and milk allowance on responses to handling, play, and social behavior in young dairy calves. *J. Dairy Sci.* **95**, 6571-6581, doi:10.3168/jds.2011-5170 (2012).

640 Miller-Cushon, E. K., Bergeron, R., Leslie, K. E., Mason, G. J. & DeVries, T. J. Competition during the milk-feeding stage influences the development of feeding behavior of pair-housed dairy calves. *J. Dairy Sci.* **97**, 6450-6462, doi:10.3168/jds.2014-8065 (2014).

641 Miller-Cushon, E. K., Bergeron, R., Leslie, K. E., Mason, G. J. & DeVries, T. J. Effect of feed presentation on feeding patterns of dairy calves. *J. Dairy Sci.* **96**, 7260-7268, doi:10.3168/jds.2013-7013 (2013).

642 Miller-Cushon, E. K. & DeVries, T. J. Effect of social housing on the development of feeding behavior and social feeding preferences of dairy calves. *J. Dairy Sci.* **99**, 1406-1417, doi:10.3168/jds.2015-9869 (2016).

643 Miller-Cushon, E. K. & DeVries, T. J. Technical note: validation of methodology for characterization of feeding behavior in dairy calves. *J. Dairy Sci.* **94**, 6103-6110, doi:10.3168/jds.2011-4589 (2011).

644 Sutherland, M. A., Worth, G. M. & Stewart, M. The effect of rearing substrate and space allowance on the behavior and physiology of dairy calves. *J. Dairy Sci.* **97**, 4455-4463, doi:10.3168/jds.2013-7822 (2014).

645 White, B. J. *et al.* Evaluation of two-dimensional accelerometers to monitor behavior of beef calves after castration. *Am. J. Vet. Res.* **69**, 1005-1012, doi:10.2460/ajvr.69.8.1005 (2008).

646 Webster, H. B. *et al.* Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. *J. Dairy Sci.* **96**, 6285-6300, doi:10.3168/jds.2012-6238 (2013).

647 Chelack, B. J., Morley, P. S. & Haines, D. M. Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrum in calves. *Can. Vet. J.* **34**, 407-412 (1993).

648 Hill, T. M., Bateman, H. G., 2nd, Aldrich, J. M., Quigley, J. D. & Schlotterbeck, R. L. Short communication: intensive measurements of standing time of dairy calves housed in individual pens within a naturally ventilated, unheated nursery over different periods of the year. *J. Dairy Sci.* **96**, 1811-1814, doi:10.3168/jds.2012-6206 (2013).

649 Waldner, C. L. & Rosengren, L. B. Factors associated with serum immunoglobulin levels in beef calves from Alberta and Saskatchewan and association between passive transfer and health outcomes. *Can. Vet. J.* **50**, 275-281 (2009).

650 Norman, L. M., Hohenboken, W. D. & Kelley, K. W. Genetic differences in concentration of immunoglobulins G1 and M in serum and colostrum of cows and in serum of neonatal calves. *J. Anim. Sci.* **53**, 1465-1472 (1981).

651 Perino, L. J., Wittum, T. E. & Ross, G. S. Effects of various risk factors on plasma protein and serum immunoglobulin concentrations of calves at postpartum hours 10 and 24. *Am. J. Vet. Res.* **56**, 1144-1148 (1995).

652 Muller, L. D. & Ellinger, D. K. Colostral immunoglobulin concentrations among breeds of dairy cattle. *J. Dairy Sci.* **64**, 1727-1730, doi:10.3168/jds.S0022-0302(81)82754-3 (1981).

653 Tyler, J. W., Steevens, B. J., Hostetler, D. E., Holle, J. M. & Denbigh, J. L., Jr. Colostral immunoglobulin concentrations in Holstein and Guernsey cows. *Am. J. Vet. Res.* **60**, 1136-1139 (1999).

654 Murray, C. F. *et al.* The effect of dystocia on physiological and behavioral characteristics related to vitality and passive transfer of immunoglobulins in newborn Holstein calves. *Can. J. Vet. Res.* **79**, 109-119 (2015).

655 Barrier, A. C., Ruelle, E., Haskell, M. J. & Dwyer, C. M. Effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. *Prev. Vet. Med.* **103**, 248-256, doi:10.1016/j.prevetmed.2011.09.001 (2012).