Should We Consider Lysophosphatidylcholines as a Potential Immunotherapy in Dairy Calves?

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Introduction

The neonatal bovine immune system is characterized as immunonaive at birth. The calf is highly dependent on maternal immunoglobulins, cytokines, and immune cells consumed in colostrum for immune protection (i.e., passive immunity). Unfortunately, poor quality, inadequate absorption, or feeding of insufficient amounts of colostrum may lead to failure of passive transfer of immunity from the dam to the calf, which causes the calf to become susceptible to early-life diseases. As a result, there is a window in time pre-weaning when the calf is highly susceptible to disease, termed the “gap in immunity” or “window of susceptibility”. During this time, the calf’s innate and adaptive immune systems (i.e., active immunity) are unable to provide sufficient immune protection in instances of pathogenic challenge (Chase et al., 2008). The development of a calfhood illness can negatively impact growth performance and potentially milk production later in life (Urie et al., 2018; Abuelo et al., 2021). Antibiotic therapy is one common approach to manage calfhood morbidity and prevent mortality; however, antibiotic use is often mismanaged and potentially contributes to the development of bacterial resistance (Langford et al., 2003; Walker et al., 2012). Consequently, there is a need for the development of safe, efficacious interventions that bolster immune function and thus protect against early-life disease in neonatal dairy calves. Non-antibiotic immunomodulators, that either enhance or suppress immune cell function, are a promising alternative to prevent or treat disease in young calves.

The delivery of the lysophospholipid lysophosphatidylcholine (LPC) is a promising potential strategy to bolster immunity and reduce antibiotic usage in young dairy cattle. In non-ruminants, bioactive LPC have been shown to modulate key bactericidal mechanisms in immune cells, such as neutrophils, and protect against morbidity and mortality caused by a systemic infection (Yan et al., 2004; Hong et al., 2010; Smani et al., 2015). While the mechanisms by which LPC causes these effects have received some attention in rodents and humans (Liu et al., 2020), our understanding of whether and how LPC influences immune function remained unexplored in dairy cattle. We aim to review immunity of the neonatal calf, LPC metabolism, and the modes of action by which LPC may modulate immunity in non-ruminants and ruminants. We also discuss a recent study at Cornell University that investigated the effects of LPC administration on bactericidal mechanisms in neutrophils isolated from neonatal Holstein heifer calves.

Bovine Neonatal Immunity and Disease

In mammals, the innate immune system is the body's first line of defense against disease (Turvey et al., 2010). It is a fast-acting and semi-specific form of immunity that is broadly distributed. Cellular components of the innate immune system include neutrophils, macrophages, dendritic cells, and natural killer cells. Neutrophils are an abundant and motile phagocyte that are “first responders” at the site of pathogen invasion. The ability of neutrophils to destroy microbes involves increases in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, hydrogen peroxide (H_2O_2) production (i.e., oxidative burst), and discharge of cytosolic granules containing proteins with bactericidal and permeabilizing properties. Macrophages encounter, identify, and engulf invading pathogens. At the surface of macrophages and dendritic cells, bacterial lipopolysaccharide (LPS) and viral double-stranded ribonucleic acid trigger ligation of toll-like receptors to stimulate type I interferon (e.g., IFNα and IFNβ) production. In turn, type I IFNs activate natural killer cells, which survey their environment with activating and inhibitory receptors, cytokine and chemokine receptors, and adhesion molecules. Natural killer cells also prevent infection by secreting pore-forming perforin and cytotoxic granzymes to lyse infected cells rapidly without antigen specificity.

Adaptive immunity is a much slower acting and longer lasting type of immunity (Turvey et al., 2010). The lymphocytes of the adaptive immune system include B cells and 3 major types of T cells including helper T cells, effector T cells, and suppressor T cells. B cells are stimulated by helper T cells to produce antibodies that will recognize pathogens and notify the phagocytes to destroy. Helper T cells stimulate cytotoxic T cells to develop, which kill the infected cells. Suppressor T cells deactivate both B and T cells. Memory B and T cells recall the antibody response to fight infection with reoccurrence.

The maternal womb is an environment that protects calves from pathogen exposure (Chase et al., 2008). Because the placenta of cows does not allow transmission of immunoglobulins from dam to fetus, the newborn calf relies on antibodies (e.g., IgG, IgA, and IgM), cytokines (e.g., interleukin-6 [IL6]), and leukocytes (i.e., T and B cells) provided in colostrum to enhance their immunologic protection. This passive immunity is important because the neonate experiences decreases in complement activity, neutrophil and macrophage activity, interferon production, natural killer cell functionality, and dendritic cell generation (Chase et al., 2008). The neonate is also born with no memory T or B cells, decreased lymphocyte responsiveness, and low antibody production (Chase et al., 2008). Neonates are dependent on the innate immune system to prime adaptive immunity. Because of finite passive immunity and slow development of active immunity, pre-weaned calves experience a high-risk “gap in immunity” or “window of susceptibility” spanning one to five weeks of age.

Calves are highly susceptible to infection because innate and adaptive immunity are underdeveloped. The consequence is diarrhea, septicemia, or bovine respiratory disease. Calf diarrhea (i.e., scouring) is a common early-life disease and cause of

mortality and economic loss for producers. Diarrhea is attributed to enteric pathogens including bacteria. Enterotoxigenic *Escherichia coli (E. coli)* is a common cause of neonatal diarrhea in farm animals (Dubreuil et al., 2016). Septicemia is a systemic infection in which bacteria and LPS enter the bloodstream. Umbilical cord infection is often the cause. Most septicemia cases occur in calves with *E. coli* infection and diarrhea, and calves with septicemia are prone to developing meningitis (inflammation of the meninges). Bovine respiratory disease is another condition caused by pathogens including viruses and bacteria (e.g., bovine respiratory syncytial virus). This disease causes pneumonia and fever. Dairy calf pneumonia most often afflicts calves from 2 to 6 months of age with peak incidence occurring at ~5 weeks of age (Ames, 1997). Previous studies estimate calfhood morbidities such as these at ~35% with incidence of diarrhea and bovine respiratory disease ranging from 10 to 35% (Waltner-Toews et al., 1986; Wells et al., 1997; Donovan et al., 1998; Hill et al., 2009). Unfortunately, morbidity increases mortality, reduces growth, and increases age and difficulty at first calving (Sivula et al., 1996; Rossini, 2004; Stanton et al., 2012). The risk for dairy calf mortality during the first year of life may range between 2 and 12% (mean of 5 to 11% based on cow parity) depending on the year, twins, region, age of calves, and management (Waltner-Toews et al., 1986; Del Río et al., 2007; Walker et al., 2012).

The industry standard to enhance calf immunity and prevent disease is to feed colostrum immediately after birth. However, failure of passive transfer of immunity occurs when calves absorb an inadequate amount of immunoglobulin caused by delayed feeding or when calves are fed low quality colostrum. The prevalence of failure of passive transfer in US dairy heifer calves is estimated at ~19% (Beam et al., 2009). Although calves with inadequate passive transfer are at heightened risk for infection, all calves are immunosuppressed and at risk for disease. The common approach to decrease calf morbidity and mortality is antibiotic administration; however, the extensive and potentially mismanaged use of antibiotics and the development of antibiotic resistant bacteria are major industry and societal concerns. Studies demonstrate that calves harbor highly resistant *E. coli* and prior systemic antibiotic therapy are associated with the fecal recovery of more resistant *E. coli* (Khachatryan et al., 2004; Berge et al., 2005. Consumers are concerned because pathogenic-resistant organisms propagated in livestock may enter the food supply (Landers et al., 2012). The development of novel non-antibiotic therapeutic tools that bolster immunity in livestock including dairy calves deserve consideration.

Lysophosphatidylcholine Metabolism and Immunomodulation

Lysophosphatidylcholines are bioactive lysophospholipids composed of a glycerol backbone, a single fatty acyl chain that varies in carbon length and saturation, and phosphocholine. Although the intestinal absorption of LPC is possible, secretory phospholipases A2 and lecithin:cholesterol acyltransferase control the cleavage of phosphatidylcholine to facilitate the endogenous production of LPC. Lysophosphatidylcholine can be converted to phosphatidylcholine via the actions of LPC acyltransferase and the availability of acyl-conenzyme A. Alternatively, LPC may

be degraded by lysophospholipases or autotaxin. In non-ruminants, the concentration and type of LPC is highly dependent upon the tissue and disease status (Liu et al., 2020). In mammals including humans and cows, the most abundant LPC are typically palmitoyl-LPC (LPC-16:0), stearoyl-LPC (LPC-18:0), oleoyl-LPC (LPC-18:1), and linoleoyl-LPC (LPC-18:2).

Lysophosphatidylcholines play many roles in regulating cellular function and disease development (see review by Liu et al., 2020). For instance, it is generally discussed that LPC are key components of bile that assist with the emulsification of neutral lipids in the intestine. LPC are also key components of cellular membranes and lipoproteins including a main constituent of oxidatively damaged low-density lipoproteins. More recently, bioactive properties of LPC have received attention. For example, LPC have been shown to modulate insulin-stimulated glucose disposal, endothelial calcium ion mobilization, cellular proliferation and apoptosis, and immune cell functionality including chemotaxis, phagocytosis, migration, and inflammation (Liu et al., 2020). In dairy cattle, our understanding of LPC is rudimentary. Our lab has discovered that circulating LPC status is lowest at parturition in dairy cattle transitioning from gestation to lactation (Rico et al., 2021), that endotoxin administration decreases circulating LPC concentrations in lactation cows (e.g., LPC-16:0, -18:0, and -18:1; McFadden et al., 2019), and extreme heat increases total and individual LPC in post-weaned Holstein calves experiencing heat stress (e.g., LPC-16:0, -18:0, and 18:2; unpublished); however, the importance of these findings remains uncertain. Because the transition cow experiences inflammation (Bradford et al., 2015), endotoxemia triggers immune activation, and heat stress is characterized by impairment of cellular immune response (Bagath et al., 2019), we aim to consider the role of LPC within bovine immunity.

Lysophosphatidylcholine and the development of sepsis in non-ruminants

In non-ruminants, a role for LPC has been considered during the development of sepsis. Septic patients have lower total plasma concentrations of LPC than healthy patients (Drobnik et al., 2003) and one corollary study suggests that circulating LPC are predictive of 28-day mortality in patients with severe sepsis (Park et al., 2014). Low circulating LPC concentrations may be due to downregulated secretory phospholipase A2 and lecithin-cholesterol acyltransferase activity (Ahn et al., 2017). These findings suggest that low LPC status enhances an individual's risk to succumbing to severe infection and increasing LPC status could be protective. This is supported by Yan and coworkers (2004). Specifically, in mice that undergo cecal ligation puncture (CLP) to induce experimental sepsis, mortality is nearly certain within ~10 d of the procedure; however, subcutaneous long-chain and saturated LPC-16:0 or LPC-18:0 effectively protect against sepsis-induced mortality caused by CLP or intraperitoneal *E. coli* administration. This response is less evident or non-existent with unsaturated or short-chain LPC (e.g., LPC-18:1 or LPC-6:0, respectively).

The ability of LPC to protect against sepsis-induced mortality appears to involve a direct role of LPC to modulate immune function. The action of LPC targets both the innate and adaptive immune systems. First, LPC triggers mechanisms that enhance phagocytic activity of neutrophils. Treatment of neutrophils with LPC-18:0 increases cytotoxic H_2O_2 production, increases phagocytic clearance of *E. coli*, and blocks neutrophil deactivation, negating the oxidative burst dysfunction often caused by experimentally-induced sepsis (Yan et al., 2004; Smani et al., 2015). Lysophosphatidylcholine therapy also inhibits the ability of LPS to induce tumor necrosis factor-α (TNFα; a key mediator of septic shock) release from neutrophils and promote mortality in mice (Yan et al., 2004). Lysophosphatidylcholine (i.e., LPC-16:0) treatment has also been shown to increase IFN-γ secretion from natural killer cells or T cells (Huang et al., 1999). Interferon-γ serves in part to activate macrophages (Ma et al., 2003). Lysophosphatidylcholines may also help promote B-cell antibody production. For example, treating human peripheral blood mononuclear cell cultures with LPC-18:0 increased immunoglobulin production (i.e., IgM, IgA, and IgG; Huang et al., 1999).

It remains unclear how LPC elicit their effects on neutrophil functionality. Lysophosphatidylcholines are hypothesized to bind to a G protein-coupled receptor found on immune cells called G2A to induce immune cell activation (Kabarowski, 2009); albeit, the anti-septic action of LPC may require G2A cooperativity with adenosine receptor type 2b (Li et al., 2019). One study found that LPC-18:0 increased bactericidal activity of neutrophils (i.e., increased cytotoxic H_2O_2 production), enhanced *E. coli* killing and blocked deactivation of neutrophils within a model of experimentally induced sepsis but were attenuated by blocking the G2A receptor with an anti-G2A antibody (Chen et al., 2005; Hong et al., 2010). Alternatively, toll-like receptors may mediate LPC action (Liu et al., 2020).

Lysophosphatidylcholine therapy also appears to attenuate the effects of infection in part by bactericidal-independent mechanisms. For example, LPC may suppress the activation and release of inflammatory elements such as high-mobility group box-1 (HMGB1) and caspase-11 (Chen et al., 2005; Li et al., 2018). High-mobility group box-1 is a ubiquitous nuclear protein secreted by monocytes and macrophages, and it is considered a stimulator of proinflammatory cytokine release from immune cells and late mediator of sepsis (Stevens et al., 2017). Injection of anti-HMGB1 antibodies, or treatment with agents that inhibit its release, such as LPC-18:0, have been found to protect mice against sepsis (Yang et al., 2004; Chen et al., 2005).

Lysophosphatidylcholine enhances bactericidal mechanisms in neutrophils isolated from pre-weaned Holstein heifer calves

There is a need to develop non-antibiotic interventions that prevent and address the development of early-life illnesses in neonatal calves in order to reduce industry antibiotic use and improve animal health. Therefore, our lab performed a study to investigate the effects of LPC on bactericidal mechanisms in neutrophils isolated from dairy calves. Polymorphonuclear leukocytes were isolated from Holstein heifer calves (2

to 5 wk of age) via Ficoll gradient double-density centrifugation and re-suspended in Roswell Park Memorial Institute (RPMI)-1640 media. The resulting cell suspension was composed of ~95% neutrophils. These neutrophils were treated in the absence or presence of 50 μM LPC-16:0, -18:0, or -18:1 in a 1:2 molar ratio with bovine serum albumin for varying lengths of time at 37°C and 5% CO_2. We performed tests to assess neutrophil functionality including H_2O_2 production to evaluate the oxidative burst, TNFα and IL6 secretion in the absence or presence of LPS (i.e., *E. coli* O55:B5), and *E. coli* killing capacity (i.e., *E. coli* cell suspensions followed by Luria broth agar plating to count colony-forming units). Statistical analyses were carried out using the mixed model procedure of SAS (v9.4, SAS Institute Inc., Cary, NC) with the model including the fixed effect of treatment and the random effect of calf and replicate within treatment. For each experiment, 3 calves were used with biological and technical replicates performed in duplicate.

We first determined that LPC did not overtly modify neutrophil viability. We then confirmed that phorbol myristate acetate (PMA), an agonist of NADPH oxidase, stimulated H_2O_2 production as quantified by a luminol chemiluminescence assay, relative to an unsupplemented control ($P < 0.01$). We then discovered that LPC-16:0 and -18:0 robustly increased H_2O_2 production, relative to unsupplemented controls ($P < 0.001$). The effect was less robust for LPC-18:1 but still significant ($P < 0.001$). We then compared the effects of LPC-18:0 versus LPC-18:1 on neutrophil TNFα and IL6 secretion in the absence of presence of LPS using ELISA. No change in cytokine secretion was observed in response to LPC in the absence of LPS; however, LPC-18:0 (but not LPC-18:1) potentiated the ability of LPS to stimulate TNFα and IL6 secretion ($P < 0.05$). Indeed, neutrophils in the absence of LPC were able to kill *E. coli* when co-cultured with live *E. coli* in a ratio of 1:10, respectively, relative to cultures with just *E. coli* ($P < 0.01$). The presence of LPC-18:0 was able to enhance the ability of neutrophils to kill *E. coli*, relative to neutrophils and *E. coli* co-cultured in the absence of LPC ($P < 0.001$). This effect was not observed for LPC-18:1. The ability of LPC-18:0 to directly kill *E. coli* in the absence of neutrophils was also investigated but proved insignificant. Collectively, our findings indicate that saturated LPC (i.e., LPC-18:0) induce neutrophil activation. It appears possible that LPC-18:0 helps neutrophils kill *E. coli* in part by inducing the oxidative burst. The ability of LPC-18:0 to induce pro-inflammatory cytokine secretion in the presence of LPS is also intriguing; however, we were unable to assess what effect this may have on immunity using this in vitro approach. It is likely that this response would elicit effects on other host immune cells. Whether the totality of responses that we observed are due to the ability of LPC-18:0 to act via the G2A receptor should be considered. Future studies that study the effects of LPC in pre-weaned Holstein dairy calves also has scientific merit. New approaches to enhance immune function in dairy cattle could be revealed.

Summary

Lysophosphatidlycholines are effective immunomodulators in non-ruminants. Science suggests that LPC acts upon both the innate and adaptive arms of the host

immune system, upregulating mechanisms involved in pathogen clearance and immune protection. In dairy cattle experiencing endotoxemia or parturition, we have revealed that circulating concentrations of LPC are low. Moreover, our in vitro data indicates that LPC do activate neutrophils isolated from pre-weaned Holstein heifer calves. We can only hypothesize that the observed increase in the oxidative burst, cytokine secretion, and *E. coli* killing have the potential to translate into heightened immune response in young calves. However, this will require careful consideration of mode of delivery, LPC type, dose, and duration. The identification of novel immunotherapies that could replace antibiotics and prevent disease deserves our attention considering the calf's susceptibility to infection and disease. The effects of LPC in older animals including periparturient and heat-stressed dairy cattle that may also experience bouts of endotoxemia also requires consideration.

References

Abuelo, A., F. Cullens, and J. L. Brester. 2021. Effect of preweaning disease on the reproductive performance and first-lactation milk production of heifers in a large dairy herd. J. Dairy Sci. 104:7008-7017.

Ahn, W. G., J. S. Jung, H. Y. Kwon, and D. K. Song. 2017. Alteration of Lysophosphatidylcholine-related metabolic parameters in the plasma of mice with experimental sepsis. Inflammation 40:537–545.

Ames, T. R. 1997. Dairy calf pneumonia: the disease and its impact. Vet. Clin. North Am. Food Anim. Pract. 13:379-391.

Bagath, M. G. Krishnan, C. Devaraj, V. P. Rashamol, P. Pragna, A. M. Lees, and V. Sejian. 2019. The impact of heat stress on the immune system in dairy cattle: A review. Res. Vet. Sci. 126:94-102.

Beam, A., J. Lombard, C. Kopral, L. Garber, A. Winter, J. Hicks, and J. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92:3973-80.

Berge, A. C. B., E. R. Atwill, and W. M. Sischo. 2005. Animal and farm influences on the dynamics of antibiotic resistance in faecal Escherichia coli in young dairy calves. Prev. Vet. Med. 69:25-38.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631-6650.

Chase, C. C. L., D. J. Hurley, and A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. North Am. - Food Anim.

Pract. 24:87–104.

Chen, G., J. Li, X. Qiang, C. J. Czura, M. Ochani, K. Ochani, L. Ulloa, H. Yang, K. J. Tracey, P. Wang, A. E. Sama, and H. Wang. 2005. Suppression of HMGB1 release by stearoyl lysophosphatidylcholine: An additional mechanism for its therapeutic effects in experimental sepsis. J. Lipid Res. 46:623–627.

Del Río, N. S., S. Stewart, P. Rapnicki, Y. M. Chang, and P. M. Fricke. 2007. An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle. J. Dairy Sci. 90:1255-64.

Drobnik, W., G. Liebisch, F.X. Audebert, D. Fröhlich, T. Glück, P. Vogel, G. Rothe, and G. Schmitz. 2003. Plasma ceramide and lysophosphatidylcholine inversely correlate with mortality in sepsis patients. J. Lipid Res. 44:754–761.

Donovan, G. A., I. R. Dohoo, D. M. Montgomery, and F. L. Bennett. 1998. Calf and disease factors affecting growth in female Holstein calves in Florida, USA. Prev. Vet. Med. 33:1-10.

Dubreuil, J. D., R. E. Isaacson, and D. M. Schifferli. 2016. Animal enterotoxigenic Escherichia coli. EcoSal Plus. 7:10.1128.

Hill, A. E., A. L. Green, B. A. Wagner, and D. A. Dargatz. 2009. Relationship between herd size and annual prevalence of and primary antimicrobial treatments for common diseases on dairy operations in the United States. Prev. Vet. Med. 88:264-277.

Hong, C.-W., T.-K. Kim, H.-Y. Ham, J.-S. Nam, Y.H. Kim, H. Zheng, B. Pang, T.-K. Min, J.-S. Jung, S.-N. Lee, H.-J. Cho, E.-J. Kim, I.-H. Hong, T.-C. Kang, J. Lee, S.B. Oh, S.J. Jung, S.J. Kim, and D.-K. Song. 2010. Lysophosphatidylcholine Increases neutrophil bactericidal activity by enhancement of azurophil granule-phagosome fusion via glycine·GlyRα2/TRPM2/p38 MAPK signaling. J. Immunol. 184:4401–4413.

Huang, Y. H., L. Schäfer-Elinder, R. Wu, H. E. Claesson, and J. Frostegård. 1999. Lysophosphatidylcholine (LPC) induces proinflammatory cytokines by a platelet-activating factor (PAF) receptor-dependent mechanism. Clin. Exp. Immunol. 116:326–331.

Kabarowski, J.H. 2009. G2A and LPC: Regulatory functions in immunity. Prostaglandins Other Lipid Mediat. 89:73–81.

Khachatryan, A. R., D. D. Hancock, T. E. Besser, and D. R. Call. 2004. Role of calf-adapted *Escherichia coli* in maintenance of antimicrobial drug resistance in dairy calves. Appl. Environ. Microbiol. 70:752-757.

Landers, T. F., B. Cohen, T. E. Wittum, and E. L. Larson. 2012. A review of antibiotic use in food animals: perspective, policy, and potential. Public Health Rep. 127:4-22.

Langford, F. M., D. M. Weary, and L. Fisher. 2003. Antibiotic resistance in gut bacteria from dairy calves: A dose response to the level of antibiotics fed in milk. J. Dairy Sci. 86:3963–3966.

Li, H.-M., J. H. Jang, J.-S. Jung, J. Shin, C. O. Park, Y.-J. Kim, W.-G. Ahn, J.-S. Nam, C.-W. Hong, J. Lee, Y.-J. Jung, J.-F. Chen, K. Ravid, H. T. Lee, W.-K. Huh, J. H. Kabarowski, and D.-K. Song. 2019. G2A protects mice against sepsis by modulating kupffer cell activation: Cooperativity with adenosine receptor 2b. J. Immunol. 202:527–538.

Li, W., W. Zhang, M. Deng, P. Loughran, Y. Tang, H. Liao, X. Zhang, J. Liu, T.R. Billiar, and B. Lu. 2018. Stearoyl lysophosphatidylcholine inhibits endotoxin-induced caspase-11 activation. Shock 50:339–345.

Liu, P., W. Zhu, C. Chen, B. Yan, L. Zhu, X. Chen, and C. Peng. 2020. The mechanisms of lysophosphatidylcholine in the development of diseases. Life Sci. 247:117443.

Ma, J., T. Chen, J. Mandelin, A. Ceponis, N.E. Miller, M. Hukkanen, G. F. Ma, and Y. T. Konttinen. 2003. Regulation of macrophage activation. Cell. Mol. Life Sci. 60:2334–2346.

McFadden, J. W., J. E. Rico, E. A. Horst, L. M. van den Brink, and L. H. Baumgard, 2019. Intravenous lipopolysaccharide infusion modifies the bovine metabolome and lipidome. J. Dairy Sci. 102(E- Suppl. 1):214. (Abstract)

Park, D. W., D. S. Kwak, Y. Y. Park, Y. Chang, J. W. Huh, C.M. Lim, Y. Koh, D. K. Song, and S.B. Hong. 2014. Impact of serial measurements of lysophosphatidylcholine on 28-day mortality prediction in patients admitted to the intensive care unit with severe sepsis or septic shock. J. Crit. Care 29:882.e5-.e11.

Rico, J. E., S. Saed Samii, Y. Zang, P. Deme, N. J. Haughey, E. Grilli, and J. W. McFadden. 2021. Characterization of the plasma lipidome in dairy cattle transitioning from gestation to lactation: Identifying novel biomarkers of metabolic impairment. Metabolites 11:290.

Rossini, K. L. 2004. Effects of calfhood respiratory and digestive disease on calfhood morbidity and first lactation production and survival rates. Virginia Polytechnic Institute and State University, Blacksburg, Virginia (Master's Thesis).

Sivula, N. J., T. R. Ames, W. E. Marsh, and R. E. Werdin. 1996. Descriptive epidemiology of morbidity and mortality in Minnesota dairy heifer calves. Prev. Vet.

Med. 27:155-171.

Smani, Y., J. Domínguez-Herrera, J. Ibáñez-Martínez, and J. Pachóna. 2015. Therapeutic efficacy of lysophosphatidylcholine in severe infections caused by Acinetobacter baumannii. Antimicrob. Agents Chemother. 59:3920–3924.

Stanton, A., D. Kelton, S. LeBlanc, J. Wormuth, and K. Leslie. 2012. The effect of respiratory disease and a preventative antibiotic treatment on growth, survival, age at first calving, and milk production of dairy heifers. J. Dairy Sci. 95:4950-4960.

Stevens, N. E., M. J. Chapman, C. K. Fraser, T. R. Kuchel, J. D. Hayball, and K. R. Diener. 2017. Therapeutic targeting of HMGB1 during experimental sepsis modulates the inflammatory cytokine profile to one associated with improved clinical outcomes. Sci. Rep. 7:1–14.

Turvey, S. E. and D. H. Broide. 2010. Innate immunity. J. Allergy Clin. Immunol. 125:S24-S32.

Urie, N., J. Lombard, C. Shivley, C. Kopral, A. Adams, T. Earleywine, J. Olson, and F. Garry. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J. Dairy Sci. 101:9229–9244.

Walker, W. L., W. B. Epperson, T. E. Wittum, L. K. Lord, P. J. Rajala-Schultz, and J. Lakritz. 2012. Characteristics of dairy calf ranches: Morbidity, mortality, antibiotic use practices, and biosecurity and biocontainment practices. J. Dairy Sci. 95:2204–2214.

Waltner-Toews, D., S. Martin, A. Meek, and I. McMillan. 1986. Dairy calf management, morbidity and mortality in Ontario Holstein herds. I. The data. Preventive Veterinary Medicine. 4:103-124.

Wells, S. J., L. P. Garber, and G. W. Hill. 1997. Health status of preweaned dairy heifers in the United States. Preventive Veterinary Medicine. 29:185-199.

Yan, J. J., J. S. Jung, J.E. Lee, J. Lee, S. O. Huh, H. S. Kim, K. C. Jung, J. Y. Cho, J. S. Nam, H. W. Suh, Y. H. Kim, and D. K. Song. 2004. Therapeutic effects of lysophosphatidylcholine in experimental sepsis. Nat. Med. 10:161–167.

Yang, H., M. Ochani, J. Li, X. Qiang, M. Tanovic, H. E. Harris, S. M. Susarla, L. Ulloa, H. Wang, R. DiRaimo, C. J. Czura, H. Wang, J. Roth, H. S. Warren, M. P. Fink, M. J. Fenton, U. Andersson, and K. J. Tracey. 2004. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. Proc. Natl. Acad. Sci. U. S. A. 101:296–301.