ABSTRACT

Dairy cattle are at the greatest risk of developing diseases around the time of calving because of compromised immune responses and the occurrence of oxidative stress. Both the development of compromised immunity and oxidative stress are influenced directly or indirectly by the metabolism of polyunsaturated fatty acids (PUFA) and fat-soluble vitamins. The cytochrome P450 (CYP450) family of enzymes is central to the metabolism of both classes of these compounds, but to date, the importance of CYP450 in the health of dairy cattle is underappreciated. As certain CYP450 isoforms metabolize both PUFA and fat-soluble vitamins, potential interactions may occur between PUFA and fat-soluble vitamins that are largely unexplored. For example, one CYP450 that generates anti-inflammatory oxylipids from arachidonic acid additionally contributes to the activation of vitamin D. Other potential substrate interactions between PUFA and vitamins A and E may exist as well. The intersection of PUFA and fat-soluble vitamin metabolism by CYP450 suggest that this enzyme system could provide an understanding of how immune function and oxidant status interconnect, resulting in increased postpartum disease occurrence. This review will detail the known contributions of bovine CYP450 to the regulation of oxylipids with a focus on enzymes that may also be involved in the metabolism of fat-soluble vitamins A, D, and E that contribute to antioxidant defenses. Although the activity of specific CYP450 is generally conserved among mammals, important differences exist in cattle, such as the isoforms primarily responsible for activation of vitamin D that makes their specific study in cattle of great importance. Additionally, a CYP450-driven inflammatory positive feedback loop is proposed, which may contribute to the dysfunctional inflammatory responses commonly found during the transition period. Establishing the individual enzyme isoform contributions to oxylipid biosynthesis and the regulation of vitamins A, D, and E may reveal how the CYP450 family of enzymes can affect inflammatory responses during times of increased susceptibility to disease. Determining the potential effect of each CYP450 on disease susceptibility or pathogenesis may allow for the targeted manipulation of the CYP450 pathways to influence specific immune responses and antioxidant defenses during times of increased risk for health disorders.

Key words: cytochrome p450, oxylipid, hydroxylase, vitamins, transition period

INTRODUCTION

The transition period of dairy cattle is characterized by an increase in the occurrence of inflammatory-based diseases such as mastitis and metritis that result in significant economic losses to dairy producers (Liang et al., 2017). Increased disease incidence during the transition period is partly due to metabolic stress, which consists of a triad of factors including oxidative stress, dysfunctional inflammatory responses, and altered nutrient metabolism (Sordillo and Raphael, 2013). During the transition period, oxidative stress results from damage to macromolecules when antioxidant defenses are inadequate to compensate for the increased production of pro-oxidants (Sordillo and Raphael, 2013). The dysfunctional inflammatory responses around the time of calving are attributed, in part, to alterations in the production of inflammatory signaling molecules, including potent lipid inflammatory mediators known as oxylipids (Boutet et al., 2003; Liu et al., 2005; Kuhn et al., 2017). Although oxylipids influence all aspects of inflammation, it is the overall profile and timing of production of anti-inflammatory and pro-inflammatory oxylipids during tissue insult that will determine the effectiveness of the inflammatory response (Sordillo, 2018). Dysfunctional inflammation may be characterized as an inability to promptly eliminate infectious pathogens leading to chronic disease or an overly robust response that can cause damage to host tissues (Mavangira and Sordillo, 2018). Relative production
of specific oxylipids is determined by several factors, including the availability of PUFA substrate; activity of the cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P450 (CYP450) enzymatic pathways; and the redox environment, which facilitates nonenzymatic production of oxylipids and modulates enzymatic activity (Sordillo, 2018). Through these factors, oxidative stress and dysfunctional inflammation can form destructive feedback loops that exacerbate the incidence and severity of dairy cattle disease. The involvement of CYP450 in both the occurrence of dysfunctional inflammation and oxidative stress may suggest an integral role in the increased risk of transition disease.

Origins of CYP450 predate pre-aerobic life allowing time for substantial diversification of individual enzyme isoforms (Wickramashighe and Villee, 1975). As CYP450 have evolved alongside almost all species, they have become integral to the metabolism of a plethora of compounds ingested or synthesized by animals. Early research into the role of CYP450 in cattle focused on their ability to either activate or break down xenobiotics, steroids, and pollutants (Balk et al., 1984; Waterman and Simpson, 1985). More recently, researchers have focused on other roles of CYP450 including contributions to immune regulation. Cytochrome P450 enzymes metabolize PUFA in 3 different ways including allylic oxidation, ω-hydroxylation, and olefin epoxidation by first activating an oxygen molecule at the heme-containing active site (Capdevila et al., 1992). The metabolism of PUFA requires NADPH₂, NADPH₂-reductase, and cytochrome b5 as cofactors. In allylic oxidation, CYP450 oxygenate arachidonic acid to form different positional isomers of hydroxyeicosatetraenoic acids (5-, 8-, 9-, 11-, 12- and 15-HETE) in reactions similar to lipoxygenases (Capdevila et al., 1982). Omega-hydroxylation of arachidonic acid occurs at terminal and subterminal carbons that are considered thermodynamically less reactive and therefore enzyme-dependent (Capdevila et al., 1992). The olefin oxidation of arachidonic acid results in the formation of epoxy fatty acids through an epoxidation reaction (Capdevila et al., 1981). The mechanism of oxidation by CYP450 may be significant during the transition period because the catalytic process generates reactive oxygen species (ROS) that can contribute to oxidative stress (Bondy and Naderi, 1994; Puntarulo and Cederbaum, 1998). Both NADPH₂ and cytochrome b5 are required for the metabolism of other compounds, including certain fat-soluble vitamins that have antioxidant functions. Moreover, NADPH₂ is required for the function of glutathione reductase in reducing oxidized glutathione. This may create competition for NADPH₂ during the transition period as PUFA substrates are increased in availability and redox balance shifts toward a greater pro-oxidant load requiring greater activity of glutathione reductase to mitigate oxidative stress (Castillo et al., 2005; Raphael et al., 2014).

Most oxylipids metabolized by CYP450 are derived from arachidonic acid and linoleic acid because of the relatively greater abundance in cell membrane phospholipids (Contreras et al., 2010). The PUFA substrate and CYP450 isoform involved determines which specific oxylipid is produced and which may have pro-inflammatory and anti-inflammatory functions. The differing contribution of each CYP450 to the overall oxylipid pool makes the activity of individual CYP450 an essential component of the overall inflammatory response.

The study of CYP450 contribution to inflammatory regulation is complicated by the significant substrate promiscuity demonstrated by most CYP450 isoforms. By having diverse substrates, some CYP450 not only produce oxylipids but contribute to the metabolism of fat-soluble vitamins, xenobiotics, and endogenous steroids. For example, CYP450 family 4 subfamily F member 2 (CYP4F2) degrades vitamin E and oxidizes arachidonic acid to form the oxylipid 20-HETE (Powell et al., 1998; Sontag and Parker, 2002). For the remainder of this review, when specific CYP450 isoforms are referenced, rather than the class as a whole, the member will be abbreviated in this manner as used with CYP4F2; namely, CYP followed by the family number (4), the subfamily letter (F), and lastly the member number (2). This substrate promiscuity creates a complicated network of substrates, enzymes, and metabolites. Within this network, vitamins D and E and potentially vitamin A share CYP450 metabolic pathways with oxylipid formation. These fat-soluble vitamins play unique and essential roles in the maintenance of transition cow health by regulating beneficial inflammatory responses and providing antioxidant defenses necessary to mitigate oxidative stress (Hogan et al., 1993; Spears, 2000; Nelson et al., 2018). The relationships between oxylipid biosynthesis and fat-soluble vitamin metabolism remains unexplored in cattle. Given the importance of oxylipids and fat-soluble vitamins in dairy cattle health, any interactions between such substrates that may affect metabolism of the other deserves research attention.

During the transition period, several CYP450 substrates associated with immune regulation are found at significantly different concentrations than other stages of lactation. Polyunsaturated fatty acid substrates and oxylipid products fluctuate significantly, both increasing and decreasing around calving compared with other stages of lactation (Kuhn et al., 2017). For decades, studies profiling CYP450 substrates such as plasma
vitamins A and E also found that systemic concentrations reach a nadir shortly after calving (Goff and Stabel, 1990). More recently, we have come to learn that vitamin D is significantly lower shortly after calving compared with the dry period as well (Holcombe et al., 2018). Typically, changes in oxylipid profiles and fat-soluble vitamin concentrations around the time of calving are discussed independently without reference to potential patterns or interactions between them. Rather, as crossover exists between oxylipid production and fat-soluble vitamin metabolism, fluctuations should be explored with the understanding that substrate competition may occur or that changes in CYP450 enzyme activity may affect multiple metabolites. The hepatic transcript expression of CYP450 involved in regulation certain oxylipids and vitamins A, D, and E indeed significantly changed around the time of calving, with specific isoforms both increasing [CYP2E1, CYP2J2 (adipose expression), CYP4F2] and decreasing (CYP2C19, CYP2U1) compared with other stages of lactation (Contreras et al., 2017; Ha et al., 2017; Haga et al., 2018). However, how these changes in expression may relate to enzymatic activity and ultimately metabolite concentration remains largely unknown (Table 1).

This review aims to detail how CYP450 may influence both oxylipid and fat-soluble vitamin production in such a way as to affect the efficiency of the inflammatory response and oxidant status. With a strong appreciation that much remains unknown, further research can uncover the pieces needed to use CYP450 intervention as a viable means to improve the health of dairy cattle.

**PUFA AND VITAMIN METABOLISM BY CYP450 EPOXYGENASES**

A hallmark of the transition period in cattle is a state of dysfunctional inflammation that underlies marked predisposition to disease (Sordillo and Raphael, 2013). The inflammatory dysfunction can be attributed, in part, to a shift in the production of oxylipids, which play crucial roles in regulating the onset and resolution of inflammation during health and disease. Oxylipids are formed through enzymatic metabolism of PUFA by the COX, LOX, and CYP450 pathways and nonenzymatically through interaction with ROS (O’Donnell et al., 2009). Oxylipids produced from the COX and LOX pathways have been extensively studied in cattle during various health disorders such as mastitis, metritis, or retained placenta (Kankofer, 2002; Herath et al., 2009; Mavangira et al., 2015). More recent studies, however, suggest significant contributions of CYP450-derived oxylipids during times when dairy cattle are most susceptible to disease. For example, CYP450-derived oxylipids were among the majority of oxylipids detected in plasma and milk during the transition period and in cows with severe coliform mastitis (Mavangira et al., 2015; Kuhn et al., 2017). Major shifts in CYP450-derived oxylipids during times of increased disease risk and mastitis pathogenesis provide the rationale to explore in more detail the potential role various CYP450 pathways may have on dairy cattle health.

As individual oxylipids may have either pro-inflammatory or anti-inflammatory characteristics, relative shifts in the production of different oxylipids can have significant effects on the inflammatory response. In general, oxylipids produced via ω-6 PUFA express pro-inflammatory activities as those derived from ω-3 PUFA are generally anti-inflammatory although exceptions to both classes exist (Gabbs et al., 2015). Interestingly, CYP450 that metabolize PUFA generally show a substrate preference for ω-3 over ω-6 PUFA (Arnold et al., 2010); however, the relative abundance of ω-6 PUFA in the diet of North American dairy cattle overcomes this preference to generally produce ω-6 derived oxylipids (Mavangira et al., 2015). The recognized substrate preference for individual CYP450 isoforms adds to the complexity of to the diverse oxylipids formed during health disorders and how their abundance and overall potency may alter the efficiency of the inflammatory response.

**Epoxygenases of Arachidonic Acid**

Many CYP450 act upon PUFA as epoxygenases. Epoxygenation occurs through an addition of an oxygen atom to a midchain double bond forming an epoxide group at that site and creating a metabolite known as an epoxy fatty acid (Oliw et al., 1982). As PUFA have several midchain double bonds, each substrate can be metabolized into a different isomer representing each midchain double bond. Interestingly, isomers are produced at unequal ratios and each isomer tends to show similar, yet not identical activities (Fer et al., 2008; Gabbs et al., 2015). Arachidonic acid having 4 double bonds is metabolized to a class of epoxyicosatrienoic acids (EET), specifically 5,6-, 8,9-, 11,12-, and 14,15-EET (Figure 1). The isomers are further metabolized by the soluble epoxide hydrolase enzyme, which adds a water molecule to each epoxy bond to generate 2 hydroxyl groups into products known as dihydroxyicosatrienoic acids that have similar but typically less potent activities (Yu et al., 2000). Although many CYP450 have epoxygenase activity, in cattle there is little information evaluating the relative contribution
<table>
<thead>
<tr>
<th>CYP450</th>
<th>Substrate</th>
<th>Gene expression change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Xenobiotics, PUFA</td>
<td>Increased in cultured bovine hepatocytes with supplementation of fish oil extracts</td>
<td>(Guruge et al., 2009)</td>
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<td>Decreased in bovine macrophage cell line by bacterial and viral PAMP</td>
<td>(Toka et al., 2019)</td>
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<td>Decreased in bovine hoof dermal cells by LPS</td>
<td>(Tian et al., 2019)</td>
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<td>CYP2C19</td>
<td>Xenobiotics, PUFA</td>
<td>Decreased in liver of cows after intramammary challenge with <em>Escherichia coli</em> or LPS</td>
<td>(Jorgensen et al., 2012)</td>
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<td>Decreased in liver of cows fed rumen-protected niacin at transition</td>
<td>(Ringseis et al., 2019)</td>
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<td></td>
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<td>Decreased in liver after calving compared with before calving</td>
<td>(Ha et al., 2017)</td>
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<td>Decreased in liver of cows after intramammary challenge of <em>E. coli</em> and LPS</td>
<td>(Jorgensen et al., 2012)</td>
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<td></td>
<td></td>
<td>Decreased in mid-lactation and late-lactation cows undergoing heat stress</td>
<td>(McCracken et al., 2015)</td>
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<tr>
<td>CYP2E1</td>
<td>Xenobiotics, PUFA, vitamin A</td>
<td>Increased in liver of calves fed colostrum compared with those fed milk replacer</td>
<td>(Kruger et al., 2005)</td>
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<td>Increased in whole blood in transgenic cattle with a <em>FAT-1</em> insertion</td>
<td>(Guo et al., 2011)</td>
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<tr>
<td></td>
<td></td>
<td>Decreased in liver of cows by intramammary challenge of <em>E. coli</em> and LPS</td>
<td>(Jorgensen et al., 2012)</td>
</tr>
<tr>
<td>CYP2J2</td>
<td>PUFA, cholecalciferol</td>
<td>Negative correlation with milk lever</td>
<td>(Pacheco et al., 2018)</td>
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<td></td>
<td></td>
<td>Positive correlation with adipose expression and both circulating free fatty acids and BHB</td>
<td>(Contreras et al., 2017)</td>
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<td></td>
<td></td>
<td>Increased in adipose tissue after calving compared with before calving</td>
<td>(Contreras et al., 2017)</td>
</tr>
<tr>
<td>CYP2U1</td>
<td>Xenobiotics, PUFA</td>
<td>Decreased in liver after calving compared with before calving</td>
<td>(Ha et al., 2017)</td>
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<tr>
<td></td>
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<td>Decreased in bovine macrophage cell line by viral PAMP</td>
<td>(Toka et al., 2019)</td>
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<tr>
<td>CYP3A4</td>
<td>Xenobiotics, PUFA, vitamin A</td>
<td>Increased in whole blood of transgenic cattle with <em>FAT-1</em> insertion</td>
<td>(Guo et al., 2011)</td>
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<td></td>
<td>(cholecalciferol)</td>
<td>Increased in the abomasum of cattle resistant to nematode infection</td>
<td>(Li et al., 2011)</td>
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<td>Decreased in mid-lactation cows undergoing heat stress</td>
<td>(McCracken et al., 2015)</td>
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<td>Decreased in bovine hoof dermal cells by LPS</td>
<td>(Tian et al., 2019)</td>
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<td>CYP4A11</td>
<td>PUFA</td>
<td>Increased at 1 and 2 wk after calving in liver of cows fed a high-energy diet during the dry period</td>
<td>(Khan et al., 2015)</td>
</tr>
<tr>
<td>CYP4F2</td>
<td>PUFA, vitamin E, vitamin K,</td>
<td>Increased in liver of cows 1 wk after calving compared with 1 wk before calving and 1 mo after calving</td>
<td>(Haga et al., 2018)</td>
</tr>
<tr>
<td></td>
<td>LTB4</td>
<td>Increased in mammary tissue 6 wk after calving compared with parturition with a nadir 1 wk before calving</td>
<td>(Haga et al., 2018)</td>
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<tr>
<td></td>
<td></td>
<td>No change in any organ after supplementation with α-tocopherol</td>
<td>(Haga et al., 2015)</td>
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1LTB4 = leukotriene B4; PAMP = pathogen-associated molecular pattern.
of specific CYP450 to the overall pool of epoxides aside from the specific isoforms CYP2J2 and CYP2C11.

Previous research on EET focused largely on investigating the vasodilatory effects in limiting cardiovascular disease in humans (Roman, 2002). Interestingly, bovine in vitro models that were used in studying the cardiovascular effects of EET dates back almost 3 decades, yet such models have rarely been applied to furthering research of cattle (Rosolowsky and Campbell, 1993). Unlike in humans, cardiovascular disease is not a significant concern for dairy cattle; however, concentrations of EET were demonstrated to shift significantly in disease conditions suggesting that they may have roles in health and disease (Mavangira et al., 2015).

Epoxyeicosatrienoic acids protect bovine endothelial cells from hypoxic injury, inflammation-induced apoptosis, and oxidative stress (Yang et al., 2001, 2007). This cellular protection was shown by the exogenous addition of EET and through cellular overexpression of CYP2J2 in bovine endothelial cell models (Yang et al., 2001, 2007). In addition, EET were shown to have direct anti-inflammatory properties through inhibition of nuclear factor-κB activation and nuclear translocation in bovine endothelial cells transfected with CYP2J2 (Node et al., 1999). In mice, such activities contributed to cardio-protection against LPS mediated cardiac injury (Dai et al., 2015). Many etiologies of mastitis, such as *Streptococcus uberis* or coliform species, cause significant tissue injury and are known for their ability to damage the endothelial barrier within the mammary gland (Ryman et al., 2015; Zhao and Lacasse, 2008).

Endothelial damage allows localized and treatable infections to become systemic, life-threatening, and unresponsive to intervention. In this context, approaches to enhance mammary or systemic EET production may reduce the incidence or severity of mastitis and prevent localized infections from becoming systemic by protecting endothelial cells from hypoxic or oxidative damage.

The detection of significant changes epoxy fatty acid concentration during the transition period in cattle may suggest a role for EET in inflammatory responses at this time as well (Kuhn et al., 2017). Although inflammation is a necessary component of transition, many animals may have aberrant inflammatory responses at this time in response to pathogen challenge (Aitken et al., 2011). Such responses can cause damaging inflammation due to slow immune responses resulting in more severe and prolonged infections in addition to greater inflammatory tissue damage (Burvenich et al., 2003; Gupta et al., 2010). In vitro, both exogenous 11,12-EET and CYP2J2 overexpression in bovine endothelial cells abolish the upregulation of vascular cell adhesion molecule 1, *VCAM-1*, after inflammatory cytokine stimulation and inhibit adherence of PMN to endothelium (Node et al., 1999; Pratt et al., 2002). By reducing the adherence of leukocytes to endothelium, EET may slow the progression of inflammation and associated production of ROS, limiting oxidative tissue injury. The anti-inflammatory activities of EET are nuanced, however. Reducing PMN migration certainly may reduce tissue damage when an infection has resolved yet constraining the inflammatory response initially may limit host defenses against pathogen challenge. In such an example, timing of production is a delineation between the potential beneficial and detrimental activities of EET.

Revealing the specific roles CYP450 epoxygenases may have in mediating inflammation offers insight into what changes in CYP450 expression and activity practically mean. In light of potential benefits of EET,
practical interventions to increase their production should be evaluated. The transcript expression and activity of CYP450 epoxygenases generally are reduced under conditions of stress and disease. For example, in LPS-treated rats, almost all epoxygenases showed reduced transcript expression in at least one major organ (Anwar-mohamed et al., 2010). In cattle, although a less complete profile of CYP450 isoforms is available, epoxygenases CYP2C19 and CYP2E1 were shown to be downregulated in bovine liver after challenge with *Escherichia coli* or LPS as CYP2C19 has additionally been shown to be reduced by heat stress (Table 1; Jorgensen et al., 2012; McCracken et al., 2015). The transition period alone in otherwise healthy dairy cows can even reduce the transcript expression of epoxygenases, as evidenced by liver CYP2C19 mRNA expression (Ha et al., 2017). Given the roles these CYP450 potentially play in reducing the inflammatory response and protecting cell viability during infection, their downregulation during stress, disease, and the transition period may be contributing to the onset, progression, and damage of disease. Interestingly, expression of CYP2J2 in adipose tissue of transition dairy cattle is increased compared with the dry period in contrast to other CYP450 that are reduced by other stressful conditions (Contreras et al., 2017). Although speculative, increased transition CYP2J2 expression coincides with increased plasma 11,12-EET and 14,15-EET, in addition to linoleic acid derived epoxy fatty acids 9,10-epoxyoctadecenoic acid (EpOME) and 11,12-EET (Contreras et al., 2017). The direct causative link between CYP2J2 expression and epoxy fatty acid formation has not been shown in cattle, yet this relationship and CYP2J2 certainly deserve greater attention.

**Epoxygenases of Linoleic Acid**

Similarly to arachidonic acid epoxygenation by CYP450, linoleic acid is assumed to be oxidized by the same CYP450 that act upon arachidonic acid, forming epoxy fatty acid diols 9,10-EpOME and 12,13-EpOME. Similarly to EET, EpOME metabolites are further hydrated by soluble epoxide hydrolase to respective dihydroxyoctadecenoic acids (DiHOME) that exhibit greater potency than their EpOME precursors (Figure 1). Although epoxygenation of arachidonic acid and linoleic acid is likely carried out by the same specific CYP450 isoforms, the ratio of each oxylipid class produced by specific CYP450 isoforms remains unknown in cattle (Moran et al., 1997). The significance of EpOME and DiHOME in cattle health is also largely unknown; however, based upon murine and in vitro models, linoleic acid derived epoxy fatty acids are assumed to be generally pro-inflammatory and cytotoxic, especially the diol isomers (Moran et al., 1997; Zheng et al., 2001). The importance of filling these gaps in knowledge is understood by recognizing that EpOME and DiHOME are detected at several orders of magnitude greater than EET and dihydroxyeicosatetraenoic acids during mastitis and in the transition period of dairy cows (Mavangira et al., 2015; Kuhn et al., 2017).

The lack of knowledge regarding EpOME and DiHOME activity and production in cattle is a clear impediment to discerning their involvement in inflammation. Given the significant shifts toward the production of linoleic acid derived CYP450 oxylipids during inflammatory events and transition, a greater research emphasis should be placed on the CYP450 mediated production of these oxylipids to understand the roles they play in health and disease of dairy cattle.

**Epoxygenases and Vitamin D**

Research into the physiological activities of vitamin D in cattle has been ongoing for almost a century with a primary focus on calcium homeostasis (Chick and Roscoe, 1926). More recent work has proposed that increasing circulating concentrations of 25-(OH)D3, the precursor metabolite to active 1,25-(OH)2D3, beyond which is required to maintain calcium homeostasis may provide further health benefits to a certain point. Poindexter et al. (2020) exemplified the effects of 25-(OH)D3 by showing that 25-(OH)D3 supplemented dairy cows, compared with those fed vitamin D3, had increased concentrations of Ca and P; increased transcript expression of interleukin-13 and inducible nitric oxide synthase; and when challenged with *Streptococcus uberis* presented less severe clinical signs. Wisneski et al. (2020), modeling the interaction between 25-(OH)D and disease, found that Michigan dairy cows with serum 25-(OH)D concentrations greater than 71.4 ng/mL at 2–10 DIM had a reduced risk of uterine disease. Cattle with serum 25-(OH)D concentrations greater than 103.4 ng/mL at dry-off and 91.1 ng/mL at close-up, however, had an increased risk for elevated urine ketone concentrations. The importance of optimal circulating 25-(OH)D3 concentrations emphasizes the need to better understand its production, specifically by a currently unknown number of CYP450 25-hydroxylases.

Vitamin D3 (cholecalciferol), either consumed through the diet or synthesized in the skin upon UV light exposure is further metabolized to 25-(OH)D3 in cattle by potentially several, or other unknown, CYP450, namely CYP2J2, CYP2R1, CYP3A4, CYP11A1, and CYP27A1 (Hymoller and Jensen, 2010; Schuster,
Determining the specific CYP450 responsible for 25-hydroxylase activity in cattle is needed as several of these CYP450 are involved in other metabolic processes.

Although the dominant 25-hydroxylase in humans is CYP2R1, evidence in cattle suggests that CYP2R1 may be a less active as a 25-hydroxylase than other CYP450 (Cheng et al., 2004; Shinkyo et al., 2004). High density genotyping by Casas et al. (2013) revealed a strong correlation between SNP within CYP2J2 and 25-(OH)D concentrations in beef calves suggesting that CYP2J2 is highly involved in the activation of vitamin D. Aiding this finding, a whole-genome sequencing scan by Pacheco et al. (2018) found only 2 CYP450 genes to be associated with milk fever incidence: CYP27A1 and CYP2J2.

As previously discussed, there is the potential for CYP2J2 to be an active epoxygenase of both arachidonic acid and linoleic acid, contributing to both anti-inflammatory and pro-inflammatory oxylipid production at a yet-unknown ratio (Figure 2). The relative substrate preference of CYP2J2 for PUFA or vitamin D₃ remains unknown as well. Known is the increase in circulating and mammary gland PUFA of transition cattle and those with severe coliform mastitis (Mavangira et al., 2015; Kuhn et al., 2017). This increase in PUFA around calving and during disease certainly may reduce the 25-hydroxylase activity of CYP2J2 through substrate competition and explain, in part, the reduction in 25-(OH)D noted in transition dairy cattle compared with the dry period (Holcombe et al., 2018).

Figure 2. Cytochrome P450 enzymes (CYP450) CYP2J2 and CYP4F2 are unique for their dual roles in metabolizing PUFA and fat-soluble vitamins. As an epoxygenase, CYP2J2 can metabolize PUFA to a mixture of pro-inflammatory epoxycosatrenocic acids (EET) and anti-inflammatory epoxygenic acids (EpOME). Additionally, CYP2F2 activates vitamin D (cholecalciferol) in the first step of the vitamin D metabolic pathway to 25-hydroxyvitamin D [25-(OH)D₃], promoting calcium homeostasis and microbial killing, as in mastitis (Lippolis et al., 2011). Similarly, CYP4F2 metabolizes arachidonic acid to the generally pro-inflammatory 20-hydroxyicosatetraenoic acid (HETE) in addition to beginning the breakdown cascade of vitamin E analogs. Because analogs of vitamin E have potent antioxidative activities, reducing the concentration of vitamin E likely reduces an animal’s antioxidant potential.
proposed activity of CYP3A4 or CYP2C11 indeed have significant 25-hydroxylase activity, increased concentrations of PUFA at certain life stages could additionally alter the activation of vitamin D.

Epoxygenases and Vitamin A

Vitamin A is an umbrella term for several metabolites, including β-carotene, retinol, and their subsequent metabolic products, such as retinoic acids (RA), that have key functions in cattle including reducing pro-oxidant load and supporting immune system maturation and function (Shi et al., 2018; McGill et al., 2019). Some studies have associated greater plasma concentrations of total vitamin A and retinol at calving or early lactation with reduced incidence of mastitis underscoring the importance of retinol at this life stage (Johnston and Chew, 1984; LeBlanc et al., 2004). The metabolic processes controlling the degradation of active vitamin A metabolites, RA, however, remain undeveloped in cattle. The CYP26 family of CYP450 isoforms is the primary pathway for metabolism of RA in humans, making them essential proteins (Thatcher et al., 2010; Ross and Zolfaghari, 2011). In cattle, apart from confirming that RA is degraded by a member of the CYP450 family, the specific CYP450 isoforms responsible for RA metabolism remains speculative (Doyle et al., 1995).

In calves, Kruger et al. (2005) provides indirect evidence that CYP26A1 is indeed responsible for at least a portion of RA metabolism. By feeding vitamin A supplement to calves and comparing their CYP450 transcript expression to a control group, it was shown that CYP26A1 was significantly increased in response to the vitamin A, likely due to it being a substrate (Kruger et al., 2005). This study additionally showed that transcripts for CYP2A6, CYP2B6, CYP2E1, CYP2C8, and CYP3A4 were not affected by vitamin A supplementation. Despite this, there is not direct evidence, such as microsomal assays, that proves or disproves that any of these CYP450, or others, have RA-hydroxylase activity.

Importantly, many of the non-CYP26 RA-hydroxylases found in humans, with potential activity in cattle, are also epoxygenases. In humans, CYP1A1, CYP2C8, CYP2C9, and CYP3A4 are involved in the metabolism of RA albeit to a lesser extent than CYP26 enzymes (McSorley and Daly, 2000; Marill et al., 2002; Ross and Zolfaghari, 2011). The potential contributions of epoxygenases to have dual roles as RA-hydroxylases should nonetheless be considered when studying both oxylipid biosynthesis and vitamin A’s contribution to dairy cattle health. Without knowing how substrates may interact, the potential remains for competition to exist between PUFA and RA.

PUFA AND VITAMIN METABOLISM BY CYP450 HYDROXYLASES

ω-Hydroxylases of Arachidonic Acid

Several CYP450 with epoxygenation activity additionally metabolize PUFA through hydroxylation reactions at the terminal or subterminal positions to form correspondingly named HETE. For example, terminal carbon hydroxylation of arachidonic acid yields 20-HETE whereas isomers of subterminal hydroxylation include 16-, 17-, 18-, and 19-HETE (Figure 1; Powell et al., 1998; Shoieb et al., 2019). This distinction is important in understanding how the relative abundances of these HETE may affect inflammatory responses due to their differing inflammatory activities. A greater body of evidence suggests that 20-HETE, for example, has pro-inflammatory activities as shown by stimulation of nuclear factor-κB in human endothelial cells (Ishizuka et al., 2008). Our studies in vitro suggest that 20-HETE may be detrimental to the integrity of the bovine vascular endothelium in conditions where it is produced in excessive amounts (Mavangira et al., 2020). Although the other metabolites of arachidonic acid ω-hydroxylation are not fully characterized, the subterminal hydroxylation metabolite 19-HETE was shown to antagonize the activities of 20-HETE in rat renal arteries (Alonso-Galicia et al., 1999).

Despite the possibility for the shifts in 20-HETE production during transition and disease in cattle to affect inflammatory regulation, the specific ω-hydroxylases responsible for its production in cattle remain to be determined. Extrapolating from human research, 2 terminal carbon ω-hydroxylases likely produce almost all 20-HETE in cattle, CYP4A11 and CYP4F2 (Powell et al., 1998). In humans, CYP4F2 is the primary enzyme responsible for the production of 20-HETE, the majority of which occurs in the kidney and liver (Powell et al., 1998). Interestingly, in cattle, CYP4A11 mRNA is expressed to a greater amount in several organs in which the 2 enzymes are found, although as of yet, no information is available as to relative pharmacokinetics of CYP4F2 and CYP4A11 (Kuhn et al., 2020). Because our study evaluated only the basal gene expression of CYP4A11 and CYP4F2 in cattle, further studies are needed to determine the production and effects of terminal ω-hydroxylation metabolites. We speculate acute inflammation and the stress of transition could result in the upregulation of these genes or increased enzyme activity based on the increased abundance of 20-HETE.
Currently, the mechanism for increased 20-HETE production in cattle, which can exceed 70-fold in the mammary gland during cases of coliform mastitis, is unknown (Mavangira et al., 2015). One explanation for increased 20-HETE is an abundance of substrate. In the report by Mavangira et al. (2015), arachidonic acid was indeed found to be at a greater concentration in the milk of cattle with coliform mastitis, however the difference between sick and healthy animals was roughly 4-fold, an order of magnitude less than the change in 20-HETE production. Another explanation, however, is a change in the expression and activity of CYP4F2 and CYP4A11. Haga et al. (2018) noted an increase in CYP4F2 mRNA expression approximately 1.5 and 2.5 greater after calving compared with the dry period in the liver and mammary tissues, respectively. This increase in CYP4F2 expression supports observations of increased 20-HETE in plasma and milk during a similar timeframe (Kuhn et al., 2017). Researchers have not yet reported potential changes in expression of CYP4A11 by lactation stage. In other species, however, evidence shows that CYP4A11 is upregulated by LPS, suggesting a potential additional contributing factor to increased 20-HETE production during coliform mastitis (Figure 3) (Anwar-mohamed et al., 2010).

As the relevance of 20-HETE in dairy cattle health continues to be uncovered, targets for manipulating its production can be translated from research models in other species. In conditions where excessive 20-HETE production may be detrimental, as our data in bovine endothelial cells suggest, strategies to mitigate production may be explored. The strategies may include antagonism of the 20-HETE receptor, targeting of the predominant CYP450 isoforms responsible for 20-HETE production, enhancing the degradation of 20-HETE, or enhancing the production of oxylipids with opposing effects. Despite lacking complete specificity, one of the most promising inhibitors is HET0016, which decreases 20-HETE production with an IC50 orders of magnitude lower than that necessary to reduce EET production (Miyata et al., 2001). Use of HET0016 in vivo reduces oxidative stress and inflammation in rodent models without apparent signs of off-target effects, yet its use has thus far only been for toxicological research in cattle (Parkinson et al., 2012; Toth et al., 2013). A more practical approach may target the production of oxylipids that have opposing effects to 20-HETE, such as the epoxygenation metabolites of arachidonic acid and ω-3 fatty acids or the subterminal ω-hydroxylation product of arachidonic acid, 19-HETE. The anti-inflammatory functions of the ω-3 epoxy fatty acids largely oppose activities exhibited by 20-HETE (Zhang et al., 2014). The oxylipid 19-HETE has also shown a proclivity for antagonizing 20-HETE (Alonso-Galicia et al., 1999). Although no current research has studied the effects of 19-HETE in cattle, in other species, it has shown to be beneficial in several disease conditions as reviewed by Shoieb et al. (2019).

In cattle, CYP1A1, a CYP450 with suspected subterminal ω-hydroxylase activity based on studies in other species, has widespread and robust transcript expression in tissues and circulating leukocytes compared with other suspected subterminal ω-hydroxylases, such as CYP1A2 or CYP2E1, making it likely a prominent CYP450 to contribute to 19-HETE production (DARwish et al., 2010; El-Sherbeni and El-Kadi, 2014; Kuhn et al., 2020). In support of its positive potential, in

Figure 3. Around the time of calving, a downregulation of cytochrome P450 enzymes (CYP450) that produce 19-hydroxyeicosatetraenoic acid (HETE) may result in a reduction in its production. An increased expression of 20-HETE-producing CYP4F2 at this same time may contribute to a state of dysfunctional inflammation during the transition period, as 20-HETE has been shown to increase reactive oxygen species and break down endothelial barrier integrity. Dysfunctional inflammation predisposes animals to disease and results in an increased prevalence of mastitis during the transition period. Mastitis, in turn, further reduces mRNA expression of 19-HETE-producing CYP450, and nonbovine studies have shown that LPS increases expression of CYP4A11, resulting in additional production of 20-HETE. This imbalance of plasma 19-HETE and 20-HETE creates a negative feedback loop, potentially furthering inflammation. Solid lines are known changes or associations in cattle, whereas dashed lines are proposed activities or associations extrapolated from other mammalian species or in vitro data.
vitro overexpression of CYP1A1 in bovine mammary epithelial cells suppressed certain pro-inflammatory effects of LPS by ameliorating increases inflammatory cytokine stimulation (Zhang et al., 2018). One explanation for this activity is the antagonistic activity of 19-HETE against 20-HETE; however, such anti-inflammatory activities may be due to EET production from CYP1A1 as well. Unfortunately, expression of CYP1A1 and other subterminal ω-hydroxylases are reduced by both viral and bacterial pathogens and during clinical mastitis (Zhang et al., 2018; Toka et al., 2019). Such reductions in transcript expression may reduce the activity of these subterminal ω-hydroxylases and enhance the activity of 20-HETE.

Terminal ω-Hydroxylases and Vitamin E

Vitamin E is a potent antioxidant essential to preventing and limiting the cellular damage of pro-oxidants. As a plasma membrane associated vitamin, vitamin E’s primary role as an antioxidant is to break lipid peroxidation chain reactions that otherwise can disrupt cellular function and lead to apoptosis (Zalkin and Tappel, 1960). By donating a hydrogen from its hydroxyl group, lipid peroxide radicals are reduced to lipid hydroperoxides that can be further reduced by glutathione. As lipids are the most susceptible macromolecule to reactive metabolite damage, adequate vitamin E to limit lipid peroxidation is essential to maintaining optimum cellular function.

The term vitamin E refers to a group of 8 vitamers with similar structure, the most biologically active and well-studied of which is α-tocopherol (αT). Although found circulating and in tissue in far lesser concentrations, the 3 other tocopherols (β, γ, and δ) and 4 tocotrienols (α, β, γ, and δ) have received greater attention recently due to their potential for potent antioxidant and anti-inflammatory activities (Jiang et al., 2000; Mazlan et al., 2006). As an ω-hydroxylase, CYP4F2 not only produces 20-HETE from arachidonic acid but is the only enzyme believed to degrade analogs of vitamin E, followed by successive steps of β-oxidation to more polar metabolites (Figure 2; Sontag and Parker, 2002). The substrate preference for non-αT analogs of vitamin E over αT shown by CYP4F2 is believed to be 1 of 2 major factors that contribute to the preservation and greater bioactivity of αT compared with other analogs, the second of which being the presence of a hepatic αT transfer protein (Sontag and Parker, 2007).

The dual activities of CYP4F2, in addition to involvement in the metabolism of vitamin K and leukotriene B4, makes understanding its role in health and disease of cattle of utmost importance. Increases in αT supplementation over the last 4 decades have overcome overt clinical deficiencies, yet oxidative stress still occurs in modern dairy cattle (Kuhn et al., 2018). Transition oxidative stress is due, in part, to a reduction in plasma αT at parturition (Goff and Stabel, 1990; Haga et al., 2018). Although supplementing ever greater concentrations of αT could be considered as a possible solution, one study supplementing αT just 3-fold greater than NRC recommendations counterintuitively reported increased oxidative stress and disease incidence (Bouwstra et al., 2010a, 2010b). Rather, increasing concentrations of non-αT analogs of vitamin E to exploit their antioxidative activities could be a future research focus. Certainly, human medical research has taken this approach to address a variety of medical conditions such as asthma, metabolic syndrome, and others as reviewed in detail by Jiang (2014), Devaraj et al. (2008), and Hernandez et al. (2013). If increasing circulating or stored concentrations of non-αT analogs is desired for dairy cattle, the activity of CYP4F2 will almost undoubtedly need to be inhibited in some manner. The aforementioned compound HET0016 may be a viable candidate for such inhibition, however, several human studies have focused on the sesame plant lignin sesamin as a more natural means to increase non-αT retention by inhibiting CYP4F2 with minimal off-target effects (Cooney et al., 2001; Frank et al., 2004; Wu et al., 2009). Use of sesamin has indeed resulted in a reduced redox state in human trials likely by reducing the clearance of tocopherols (Barbosa et al., 2017).

The importance of further research into the activities of 20-HETE is bolstered by the potential for inhibition of CYP4F2 to both increase vitamin E concentrations and reduce 20-HETE. Currently, as the activities of 20-HETE are understood, reducing its production may reduce inflammation and limit tissue damage from dysfunctional inflammatory responses at transition. However, it is unknown if reducing 20-HETE may also hamper the necessary inflammatory response to pathogen challenge. Until the activities of 20-HETE can be resolved, potentiating the activity of CYP4F2 could cause unintended negative consequences by constraining necessary inflammatory cascades.

CONCLUSIONS AND FUTURE DIRECTIONS

Advances in research of oxylipid production and fat-soluble vitamin metabolism have begun to uncover the complexity and importance of CYP450 to the maintenance of dairy cattle health. Despite this, published descriptions of CYP450 activities in cattle are largely extrapolated from human medicine or other mammalian research with limited work devoted to discerning species-specific differences. Although CYP450 activity is generally conserved among species, exceptions cer-
certainly exist. Importantly, evidence in cattle suggests that CYP2J2 is a physiologically relevant enzyme to produce 25-(OH)D₃, unlike humans that rely upon CYP2R1 for 25-hydroxylase activity. Recognizing this role of CYP2J2 is essential for developing studies that consider its contribution to the vitamin D cascade and interpret changes in 25-(OH)D concentration within the context of PUFA availability. Additionally, RA-hydroxylase activity of CYP450 in cattle is assumed to be carried out by the CYP26 family of enzymes, however, it is unknown if non-CYP26 enzymes may contribute as well and if so, to what relative extent. Lastly, CYP4F2 has underappreciated activities in regulating inflammation and redox balance by producing the potent oxylipid 20-HETE and degrading analogs of vitamin E. Until a greater understanding of 20-HETE and its benefits or detriments to transition cow health is developed, the potential for inhibition of CYP4F2 as a therapeutic target will remain unknown.

Broadly, research into the manipulation of CYP450 may serve to reduce the generally pro-inflammatory oxylipid profile produced by modern dairy cattle due to the preponderance of ω-6 PUFA in their diet. Rather than dramatically shifting the diet of cattle away from ω-6 PUFA or supplementing significant amounts of ω-3 PUFA, altering the activity of specific CYP450 may reduce the pro-inflammatory nature of oxylipid production. Increasing the activity of CYP450 such as CYP2J2 or CYP1A1 while reducing the activity of the 20-HETE producing enzymes CYP4F2 and CYP4A11 could dramatically tip the inflammatory scales toward a more balanced phenotype. Although pro-inflammatory oxylipids are certainly necessary for appropriate immune responses to pathogen challenge, a reduction in the abundance of pro-inflammatory oxylipids could reduce tissue damage caused by overly robust, unresolved, and dysfunctional inflammatory responses noted during many transition period infections.

Not discussed in this review is the significant contribution of CYP450 to endogenous steroid production, such as the synthesis of precursors to cortisol, or contributions to the metabolism of xenobiotics necessary for the bioactivation or breakdown of many pharmaceutical interventions (Niwa et al., 2015; Rekka et al., 2019). Nonetheless, CYP450 that participate in these metabolic pathways are further intertwined with those metabolizing fat-soluble vitamins and producing oxylipids. For example, CYP1A1 and CYP3A4 are believed to both be epoxygenases yet carry out metabolism of many xenobiotics (Burke et al., 1994; Fer et al., 2008).

Despite the many potential uses for inhibitors of specific CYP450 to prevent or treat disease, research into their use has been fraught with unintended off-target effects due to the complexity of CYP450 metabolism. Many inhibitors that potently inhibit a single CYP450 still exert partial inhibition of others. Additionally, the substrate multiplicity of CYP450 means that inhibiting a CYP450 isoform with multiple substrates may affect several metabolic pathways. Although the use of specific inhibitors may pose challenges, there is a potential for therapeutic intervention using CYP450 inhibitors. Although this review focused on only 2 CYP450 with known dual activities between PUFA and fat-soluble vitamins, CYP2J2 and CYP4F2, there are significant gaps in our knowledge of the CYP450 family of oxylipids and other dual activity CYP450 likely exist. The potential for such enzymes is exemplified by the likelihood of additional RA-hydroxylases with a dual role as an epoxygenase or in xenobiotic metabolism. As research into CYP450 advances, discovering and understanding CYP450 involved in multiple metabolic cascades affecting dairy cattle health can help to identify potential candidate enzymes to intervention that may provide benefits to health through multiple substrate pathways.

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