Leaky Gut's Contribution to Inefficient Nutrient Utilization

E.J. Mayorga1, E.A. Horst1, S.K. Kvidera1, J. Ydstie, M. Al-Qaisi1, M.J. Dickson1, C.S. Shouse, H.R. Ramirez, R.P. Rhoads2, A.F. Keating1, R. Appuhamy1 and L.H. Baumgard1

1Iowa State University Department of Animal Science
2Virginia Tech Department of Animal Science
Corresponding author: baumgard@iastate.edu

Introduction

There are a variety of situations in an animal's life when nutrient utilization is reprioritized from productive towards agriculturally unproductive purposes. Two well-known examples that markedly reduce production are heat stress and ketosis. Decreased feed intake, experienced during both diseases, is unable to fully explain decreases in productivity. Additionally, both diseases are characterized by negative energy balance, body weight loss, inflammation, and hepatic steatosis. While the metabolism of ketosis and heat stress have been thoroughly studied for the last 40 years, the initial insult in the cascade of events ultimately reducing productivity in both heat-stressed and ketotic cows has not been identified. To that end, we have generated preliminary data strongly implicating a metabolic disruptor, endotoxin, as the etiological culprit in each case.

Heat Stress

Heat stress negatively impacts a variety of production parameters and is a significant financial burden (~$900 million/year for dairy in the U.S. alone; St. Pierre et al., 2003). Heat-stress affects productivity indirectly by reducing feed intake; however, direct mechanisms also contribute as we have shown reduced feed intake only explains approximately 35-50% of the decreased milk yield during heat stress (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). Direct mechanisms contributing to heat stress milk yield losses involve an altered endocrine profile, including reciprocal changes in circulating anabolic and catabolic hormones (Bernabucci et al., 2010; Baumgard and Rhoads, 2012). Such changes are characterized by increased circulating insulin concentration, lack of adipose tissue lipid mobilization, and reduced adipocyte responsiveness to lipolytic stimuli. Hepatic and skeletal muscle cellular bioenergetics also exhibit clear differences in carbohydrate production and use, respectively, due to heat stress. Thus, the heat stress response markedly alters post-absorptive carbohydrate, lipid, and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner distinct from commonly recognizable changes that occur in animals on a reduced plane of nutrition (Baumgard and Rhoads, 2013). The result of HS is underachievement of an animal's full genetic potential.

Ketosis

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptations to support milk production. Unfortunately, a disproportionate amount of herd culling occurs before cows reach 60 days in milk (Godden, 2003). Ketosis is defined as an excess of circulating ketone bodies and is characterized by decreases in feed intake, milk production, and increased risk of developing other transition period diseases (Chapinal et al., 2012). Epidemiological data indicate about 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and thus it represents a major hurdle to farm profitability. Traditionally, ketosis is thought to result from excessive adipose tissue mobilization (Baird, 1982; Grummer, 1993; Drackley, 1999) which in turn contributes to fatty liver (hepatic steatosis) and excessive ketone body synthesis (Grummer, 1993).

Heat stress etiology

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health and function as we and others have demonstrated HS compromised intestinal barrier function (Lambert et al., 2002; Dokladny et al., 2006; Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat leading to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP deple-

tion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013). As a result, HS increases the passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as lipopolysaccharide (LPS), is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Activation of the immune system occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and TLR4 delivers LPS for removal and detoxification, thus LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our recent review (Mani et al., 2012). Endotoxin infiltration during HS into the bloodstream which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10 fold increase in circulating insulin (Rhoads et al., 2009; Stoakes et al., 2015a; Kvidera et al., 2016). Interestingly, increased insulin occurs prior to increased inflammation and the temporal pattern agrees with our previous in vivo data and a recent in vitro report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of heat-stressed agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin in both models is energetically difficult to explain as feed intake was severely depressed in both experiments.

Transition period inflammation

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Ametaj et al., 2005; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Loor et al., 2005; Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and postpartum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (Fig. 1; Abuajamieh et al., 2015). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis), mammary gland (mastitis) and the gastrointestinal tract (Mani et al., 2012). However, we believe intestinal permeability may be responsible for inflammation observed in the transition dairy cow. A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage based to a high concentrate ration. This has the potential to induce rumen acidosis which can compromise the gastrointestinal tract barrier (Khafipour et al., 2009).

In order to further investigate the effects of intestinal permeability on production and inflammation, we intentionally induced intestinal permeability in mid-lactation dairy cows using a gamma secretase inhibitor (GSI), a compound that specifically inhibits crypt stem cell differentiation into enterocytes via disrupting Notch signaling (van Es et al., 2005). We anticipated feed intake of GSI administered cows would decrease, so we pair-fed controls in order to eliminate the confounding effect of feed intake. Treatment with GSI decreased feed intake and altered jejunum morphology consistently with characteristics of leaky gut (shortened crypt depth, decreased villus height, decreased villus height to crypt depth ratio). Circulating insulin and LBP were increased in GSI cows relative to controls. Interestingly in our GSI model, acute phase proteins serum amyloid A and haptoglobin increased for both treatments over time, indicating inflammation was occurring in pair-fed controls as well (Kvidera et al., 2017a). This is not surprising, as pair-fed controls were receiving ~20% of their ad libitum intake and decreased feed intake has been shown to increase intestinal permeability in feed restricted rodents and humans (Rodriguez et al., 1996; Welsh

et al., 1998) and we have also observed this in pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Recently, we confirmed the detrimental effects of feed restriction in mid-lactation cows by demonstrating a linear increase in circulating acute phase proteins and endotoxin with increasing severity of feed restriction. Furthermore, cows fed 40% of ad libitum intake had shortened ileum villous height and crypt depth, indicating reduced intestinal health (Stoakes et al., 2015b). In summary, inflammation is present during the transition period and likely contributes to changes in whole-animal energetics.

Metabolism of inflammation

LPS-induced inflammation has an energetic cost which redirects nutrients away from anabolic process that support milk and muscle synthesis (see review by Johnson, 1997, 1998) and thus compromises productivity and efficiency. Interestingly, immune cells become more insulin sensitive and consume copious amounts of glucose upon activation in order to support rapid proliferation and biosynthetic processes (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In contrast, inflammation induces an insulin resistant state in skeletal muscle and adipose tissue (Liang et al., 2013; Poggi et al., 2007). Recent data has also demonstrated a decrease in ketone oxidation during LPS infiltration (Suagee et al., 2011; Frisard et al., 2015) which we believe may partly explain increased ketone body concentrations during the transition period.

Endotoxin has previously been recognized to be involved with metabolic dysfunction. In humans, both obesity and high fat diets are linked to endotoxemia (Cani et al., 2007, Gregor and Hotamisligil, 2011). Furthermore, LPS is involved with the development of fatty liver (Ilan, 2012), and cytokines are linked to lipid accumulation and cholesterol retention (Ma et al., 2008; Clément et al., 2008). Experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and endocrine disturbances including decreased circulating glucose, termination of pregnancy, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Giri et al., 1990; Waldron et al., 2003; Jing et al., 2014). The aforementioned pathological conditions are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning caused by immune system activation.

Energetic cost of immune activation

An activated immune system requires a large amount of energy and the literature suggests that glucose homeostasis is markedly disrupted (Leininger et al., 2000) during an endotoxin challenge. Upon immune system activation, immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis, causing them to become obligate glucose utilizers in a phenomenon known as the Warburg Effect (Vander Hiden et al., 2009). Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by the immune system during a 12 hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in lactating cows, growing steers and growing pigs were 0.64, 1.0, and 1.1 g glucose/kg BW0.75/h, respectively; Stoakes et al., 2015a; Kvidera et al., 2016, 2017b). Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool). We and others have now demonstrated that both heat-stressed and ketotic animals have increased circulating markers of endotoxin and inflammation. We believe that the circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

Nutritional strategies: the role of chromium (Cr) supplementation

Potential mitigation strategies during inflammatory conditions are currently of great interest; one such strategy is dietary Cr supplementation. Chromium is a nutrient well known for its role in improving insulin action. While the exact mechanism is not fully understood it appears to work intimately with the oligopeptide low-molecular-weight Cr-binding substance or chromodulin. In response to increasing insulin, Cr ions enter insulin dependent cells and in combination with chromodulin bind to insulin receptor sites amplifying the signaling cascade (Vincent, 2015). Subsequently, translocation of the insulin dependent glucose transporter GLUT-4 to the plasma membrane is improved and cellular glucose uptake is increased (Chen et al., 2006). Glucose availability to activated leukocytes impacts apoptotic rate, formation of reactive oxygen species, and adhesion interactions (Calder et al., 2007; MacIver et al., 2008). Previous studies have shown improved immune function (e.g., phagocytosis, blastogenic response, antibody production) of activated leukocytes supplemented with Cr (Moonsier-Shageer and Mowat, 1992; Chang

et al., 1996; Lee et al., 2000), while others observed no effect (Kegley et al., 1997a). Several studies have demonstrated increased markers of cytokine production in mice, steers, and cows (Burdick et al., 2011; Yuan et al., 2014; Jin et al., 2016). Additionally, we and others have shown an impact of Cr on neutrophil proliferation (Horst et al., unpublished data; Kafilzadeh et al., 2012; Yasui et al., 2014; Mayorga et al., 2016). However, the direct influence of Cr on bovine immunity remains poorly understood and warrants further investigation.

The benefits of Cr are not limited to its influence on immunity, but extend to metabolism and production parameters as well. Previous studies in cattle have demonstrated that Cr supplementation improved glucose clearance rate and decreased circulating insulin levels following an intravenous glucose tolerance test (Bunting et al., 1994; Kegley et al., 1997b; Stahlhut et al., 2006; Summer et al., 2007; Spears et al., 2012); suggesting enhanced tissue insulin sensitivity in animals fed Cr compared to those not supplemented. Additionally, supplemental Cr consistently increases feed intake in a variety of species (Mooney and Crowell, 1997; Sahin et al., 2003; Al-Saiyadi et al., 2004; MacNamara and Valdez, 2005; Toghyani et al., 2006; Hung et al., 2014). Further, beneficial effects of dietary Cr on milk production, average daily gain, carcass traits, and reproductive parameters have been documented within the literature (Page et al., 1993, Lindemann et al., 1995; Hayirli et al., 2001; Bryan et al., 2004; Smith et al., 2005, 2008; Sadri et al., 2009; Yasui et al., 2014, Liu et al., 2017). A summary of the effects of Cr supplementation on production parameters across different species is presented in Table 1.

Conclusion

Ketosis and heat stress are two of the most economically important pathologies which severely jeopardize the competitiveness of animal agriculture. Heat stress and ketosis affect herds of all sizes and every dairy region in country. The biology of ketosis and heat stress has been studied for almost a half century, but the negative impacts of both are as severe today as they were 30 years ago. We suggest, based upon the literature and on our supporting evidence, that LPS is the common culprit etiological origin of both metabolic disorders. Taken together, our data and the literature suggest that LPS markedly alters nutrient partitioning and is a causative agent in metabolic disruption during heat stress and ketosis. Identifying nutritional strategies to improve animal welfare and performance is critically important. The use of dietary Cr as a supplement may represent a practical avenue to maximize the animal response under these circumstances.

REFERENCES

Abuajamieh, M. et al. 2016. Res. Vet. Sci. 109:81-85.
Al-Saiady, M. Y. et al. 2004. Anim. Feed Sci. Technol. 117:223-233.
Ametaj, B. N. et al. 2005. Can. J. of Anim. Sci. 85:165-175.
Amoikon, E. K. 1995. J. Anim. Sci. 73:1123-1130.
Baird, G. D. 1982. J. Dairy Sci. 65:1-10.
Baumgard, L. and R. P. Rhoads. 2013. Annu. Rev. Anim. Biosci. 1:7.1-7.27.
Baumgard, L. H. and R. P. Rhoads. 2012. J. Anim. Sci. 90:1855-1865.
Baumgard, L. H. et al. 2011. J. Dairy Sci. 94:5620-5633.
Berczi, I. et al. 1966. Can. J. of Microb. 12:1070-1071.
Bernabucci, U. et al. 2010. Animal. 4:1167-1183.
Bertoni, G. et al. 2008. J. Dairy Sci. 91:3300-3310.
Bhat, U. G. et al. 2014. J. Periodontol. 85:1629–1636.
Bradford, B. J. et al. 2009. J. Nutr. 139:1451–1456.
Bryan, M. A. et al. 2004. J. Dairy Sci. 87:4269-4277.
Bunting, L. D. et al. 1994. J. Anim. Sci. 72:1591-199.
Burdick, N. C. et al. 2011. Innate Immun. 18:592-601.
Bynum, G. et al. 1979. Aviat. Space Environ. Med. 50:816-819.
Calder, P. C. et al. 2007. Curr. Opin. Clin. Nutr. Metab. Care 10:531-540.
Cani, P. D. et al. 2007. Diabetes 56:1761-1772.
Ceciliani, F. et al. 2012. J. Proteomics 75:4207-4231.
Chang, X. et al. 1996. Vet. Immunol. Immunop. 52:37-52
Chapinal, N. et al. 2012. J. Dairy Sci. 95:5676-5682.
Chen, G. et al. 2006. Mol. Endocrinol. 20:857-870
Clément, S. et al. 2008. Hepatology 48:799-807.
Dokladny, K. et al. 2006. Am. J. Physiol. Gastrointest. Liver Physiol. 290: G204-G212.
Drackley, J. K. 1999. J. Dairy Sci. 82:2259-2273.
Frisard, M. I. et al. 2015. Metabolism 64:416-427.
Gathiram, P. et al. 1987. Circ. Shock 23:157-164.
Gillund, P. et al. 2001. J. Dairy Sci. 84:1390-1396.
Giri, S. N. et al. 1990. Vet. Microbiol. 21:211-231.
Godden, S. M. et al. 2003. Proc. Four-State Nutrition Conference. Pp. 55-64.
Graber, C. D. et al. 1971. JAMA 216:1195-1196.
Gregor, M. F. and G. S. Hotamisligil. 2011. Rev. Immunol. 29:415–445.
Griel, L. C. et al. 1975. Can. J. Comp. Med. 39:1-6.
Grummer, R. R. 1993. J. Dairy Sci. 76:3882-3896.
Hall, D. M. et al. 1999. Am. J. Physiol. 276:G1195-G1203.
Hall, D. M. et al. 2001. Am. J. Physiol. Heart Circ. Physiol. 280:H509-521.
Harper, A. F. et al. 1995. J. Anim. Sci. 73 (Suppl.):194(Abstr.)
Hayirli, A. D. et al. 2001. J. Dairy Sci. 84:1218-1230.
Humblet, M. F. et al. 2006. Vet. Clin. Pathol. 35:188-193.

Hung, A. T. et al. 2014. Trop. Anim. Health. Prod. 46:1483-1489.
Ilan, Y. 2012. World J. Gastroenterol. 18:2609-2618.
Jackson, a. R. et al. 2009. J. Anim. Sci. 87:4032-4041.
Jin, Y., et al. 2016. Chemosphere. 148:436-443.
Jing, L. et al. 2014. Br. J. Nutr. 112:170-182.
Johnson, R. W. 1997. J. Anim. Sci. 75:1244-1255.
Johnson, R. W. 1998. Domest. Anim. Endocrinol. 15:309-319.
Kafilzadeh, F. Biol. Trace. Elem. Res. 149:42-49
Kahles, F. et al. 2014. Diabetes 63:3221-3229.
Kegley, E. B. et al. 1997a. J. Dairy Sci. 75:1956-1964.
Kegley, E. B. et al. 1997b. J. Dairy Sci. 80:1744-1750.
Khafipour, E. et al. 2009. J. Dairy Sci. 92:1060-1070.
Kvidera, S. K. et al. 2017a. J. Dairy Sci. 100:4113-4127.
Kvidera, S. K. et al. 2017b. J. Dairy Sci. 100:2360-2374
Kvidera, S. K. et al. 2016. J. Anim. Sci. 94:4592-4599.
Lambert, G. P. et al. 2002. J. Appl. Physiol. 92:1750–1761.
Lee, D. N. et al. 2000. J. Anim. Sci. 13:528-234.
Leininger, M. T. et al. 2000. Domest. Anim. Endocrinol. 18:71-82.
Leon, L. R. 2007. Prog. Brain Res. 162:481-524.
Liang, H. et al. 2013. PLoS One 8:e63983.
Lim, C. L. et al. 2007. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R186-194.
Lindemann, M. D. et al. 1995. J. Anim. Sci. 73:457-465.
Liu, F. et al. 2017. Transl. Anim. 1:116-125.
Loor, J. J. et al. 2005. Physiol. Genomics 23:217–226.
Ma, K. L. et al. 2008. Hepatology 48:770-781.
Maclver, N. J. 2008. J. Leuk. Biol. 84:949-957.
Mani, V. et al. 2012. J. Anim. Sci. 90:1452-1465.
Martel, C. A. et al. 2014. J. Dairy Sci. 97:4897-4906.
Matthews, J. O. 2003. J. Anim. Sci. 81:191-196.
Matthews, J. O. 2001. J. Anim. Sci. 79:2172-2178.
Mayorga, E. J. et al. 2016. J. anim. Sci. 64(Suppl. 2):143 (Abstr.).
McArt, J. A. et al. 2012. J. Dairy Sci. 95:5056-5066.
McArt, J. A. et al. 2015. J. Dairy Sci. 98:2043-2054.
McNamara J. P., and F. Valdez. 2005. J. Dairy Sci. 88:2498-2507.
Mooney, K. W., and G. L. Cromwell. 1997. J. Anim. Sci. 75:2661-2671.
Mooney, K. W., and G. L. Cromwell. 1995. J. Anim. Sci. 73:3351-3357.
Moonsier-Shageer and Mowat. 1993. J. Anim. Sci. 71:232-238.
Mullins, C. R. 2012. J. Dairy Sci. 95:1323-1336.
Page, T. G. et al. 1993. J. Anim. Sci. 71:656-662.
Palsson-McDermott, E. M. and L. A. O'Neill. 2013. Bioessays. 35:965-973.
Pearce, S. C. et al. 2013. J. Anim. Sci. 91:5183-5193.
Poggi, M. 2007. Diabetologia 50:1267-1276.
Rhoads, M. et al. 2009. J. Dairy Sci. 92:1986-1997.
Rodriguez, P. et al. 1996. Gut 39:416–422.
Rollwagen, F. M. et al. 2006. Biochem. Biophys. Res. Commun. 347:1094-1098.
Sadri, H. et al. 2009. J. Dairy Sci. 92:5411-5418.
Sahin, K. et al. 2002. Boil. Trace elem. Res. 89:53-64.
Sahin, K. et al. 2003. Nutr. Res. 23:225-238.
Sands, J. S., and M. O. Smith. 1999. J. Appl. Poult. Res. 8:280-287.
Sanz-Fernandez, M. V. et al. 2014. Animal 8:43-50.
Smith, K. L. et al. 2008. J. Dairy Sci. 91:2011-2020.
Smith, K. L. et al. 2005. J. Dairy Sci. 88:225-263.
Soltan, M. A. et al. 2010. J. Anim. Physiol. Anim. Nutr. (Berl). 94:264-272.
Spears, J. W. et al. 2012. J. Dairy Sci. 95:2037-2045.
Stahlhut et al., 2006. Anim. Feed Sci. Technol. 128:253-265.
St. Pierre, N. et al. 2003. J. Dairy Sci. 86:E52-E77.
Stoakes, S. K. et al. 2015a. J. Anim. Sci. 93:634 (Abstr.).
Stoakes, S. K. et al. 2015b. J. Dairy Sci. 98:274 (Abstr.).
Suagee, J. K. et al. 2011. J. Vet. Intern. Med. 25:356-364.
Summer, J. M. et al. 2007. J. Dairy Sci. 90:3467-3474.
Toghyani, M. et al. 2006. Int. J. Poult. Sci. 5:65-69.
Tough, D. F. et al. 1997. J. Exp. Med. 185:2089-2094.
Uyanik, F. et al. 2002. Biol. Trace. Elem. Res. 90:99-115.
Van Es, J. H. et al. 2005. Nature 435:959–963.
Vander Heiden, M. G. et al. 2009. Science. 324:1029-1033.
Vincent, J. B. 2015. Biol. Trace Elem. Res. 166:7-12.
Waldron, M. R. et al. 2006. J. Dairy Sci. 89:596-610.
Waldron, M. R. et al. 2003. J. Dairy Sci. 86:3440-3446.
Welsh, F. K. 1998. Gut 42:396-401.
Wheelock, J., R. et al. 2010. J. Dairy Sci. 93:644-655.
Yasui T. et al. 2014. J. Dairy Sci. 97:6400-6410.
Yuan, K. et al. 2013. PloS One e80316.
Yuan, K. et al. 2014. J. Dairy Sci. 97:3822-3831.
Zhang, H. et al. 2011. Boil. Trace elem. Res. 141:159-169

Table 1. Effects of Cr supplementation on production parameters and metabolism in various species

Variable	Species	Response	Reference
Feed Intake	Cattle	=/↑	3,22/1,4,10,19,20, 21
	Pigs	=/↓/↑	7,13,12,27/11/6,14
	Poultry	↓/↑	24/16,17,23
Average Daily Gain	Cattle	↑	8,9
	Pigs	=/↑	2,12,15/5,13,26
	Poultry	↑	17,18,23
Milk yield	Cattle	=/↑	3,25/1,4,10,19,21

[1]Al-Saiyadi et al., 2004
[2]Amoikon et al., 1995
[3]Bryan et al., 2004
[4]Hayirli et al., 2001
[5]Harper et al., 1995
[6]Hung et al., 2014
[7]Jackson et al., 2009
[8]Kegley et al., 1997a
[9]Kegley et al., 1997b
[10]MacNamara and Valdez, 2005
[11]Mathews et al., 2001
[12]Mathews et al., 2003
[13]Mooney and Crowell.1995
[14]Mooney and Crowell, 1997
[15]Page et al., 1993
[16]Sahin et al., 2002
[17]Sahin et al., 2003
[18]Sands and Smith, 1999
[19]Smith et al., 2005
[20]Smith et al., 2008
[21]Soltan et al., 2010
[22]Spears et al., 2012
[23]Toghyani et al., 2006
[24]Uyanik et al., 2002
[25]Yasui et al., 2014
[26]Zhang et al., 2011

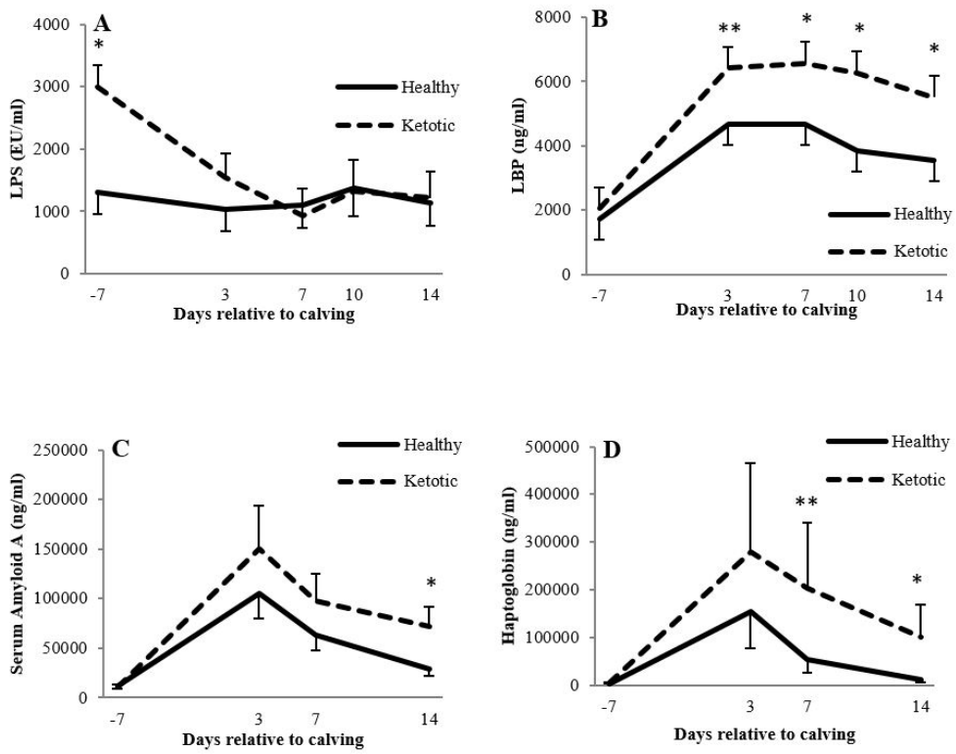

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.