RECENT RESEARCH ON HYPOCALCEMIA AND IMMUNITY

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The transition from pregnant, non-lactating to non-pregnant, lactating is too often a disastrous experience for the cow. Most of the metabolic diseases of dairy cows - milk fever, ketosis, retained placenta, and displacement of the abomasum - occur within the first 2 wk of lactation. In addition to metabolic disease, the majority of infectious disease experienced by the dairy cow, especially mastitis and metritis, but also diseases such as Johne's disease and Salmonellosis, become clinically apparent during the first 2 wk of lactation.

DOES MILK PRODUCTION AFFECT IMMUNE STATUS?

Neutrophil and lymphocyte function is diminished in the periparturient period, especially in the dairy cow (Kehrli et al., a,b). The onset of milk production imposes tremendous challenges to the mechanisms responsible for energy, protein, and mineral homeostasis in the cow. Negative energy, protein, and/or mineral balance associated with the onset of lactation may be partially responsible for the immunosuppression observed in periparturient dairy cattle. Mastectomy of pregnant dairy cows removes the impact of milk production while presumably maintaining endocrine and other changes associated with late pregnancy and parturition. Mastectomy would be expected to improve immune function in the periparturient dairy cow, if milk production is an immunosuppressive factor. Using 10 mastectomized and 8 intact multiparous Jersey cows (all intact cows developed milk fever) we assessed the ability of neutrophils to kill microbes as assessed by neutrophil myeroperoxidase activity during the periparturient period (Kimura et. al., 1999). Neutrophil myeroperoxidase activity decreased equally before parturition in both groups. While there was a quick recovery of neutrophil myeloperoxidase activity in mastectomized cows, there was no recovery in intact cows after parturition throughout the study, which lasted until d 20 post partum. Lymphocyte production of gamma-interferon in vitro declined significantly at parturition in intact cows but did not decrease significantly in the mastectomized cows. In intact cows, all T cell subset populations (i.e. CD3, CD4, CD8 and gamma-delta positive cells) decreased as a percentage of total PBMC at the time of parturition while the percentage of monocytes increased. These population changes have previously been shown to be associated with the immune suppression commonly observed in periparturient cows. Mastectomy eliminated these changes in leukocyte subsets (Kimura et al., submitted for publication). These results suggest: 1) the mammary gland may produce substances which directly affect immune cell populations, or 2) metabolic demands associated with the onset of lactation negatively impact the composition of circulating PBMC populations. Two metabolic factors were greatly impacted by mastectomy. Mastectomy eliminated hypocalcemia at parturition. Plasma Non-esterified fatty acid (NEFA) concentration rose dramaticly in intact cows at calving and

did not return to baseline level for > 10 d. In contrast NEFA concentration in mastectomized cow plasma rose only slightly at calving and returned to baseline level 1-2 days after calving.
It is clear that the intact cow mobilizes a much larger amount of body fat than does the mastectomized cow, suggestive of a severe negative energy balance at the onset of lactation.

HYPOCALCEMIA AND MASTITIS SUSCEPTIBILITY

Calcium is necessary for proper contraction of muscle. Severe hypocalcemia prevents skeletal muscle contraction to the point that the clinical syndrome known as milk fever occurs. Muscle contraction is reduced by any decrease in blood calcium, however it must be severe before we observe the “downer cow”. Daniel et al., (1983) demonstrated that contraction rate and strength of the smooth muscle of the intestinal tract is directly proportional to blood calcium concentration. The teat sphincter consists of smooth muscle which must contract if closure of the teat end is to occur. If low blood calcium reduces teat sphincter contraction the teat canal may remain open inviting environmental pathogens to enter the mammary gland. We generally associate milk fever with the day of calving but we have demonstrated that many cows remain subclinically hypocalcemic for the first week of lactation (Goff et al., 1996). Hypocalcemic cows tend to spend more time lying down than do normocalcemic animals. Again this could increase teat end exposure to environmental opportunists.

Hypocalcemia also acts as a stressor to the cow. Cows typically exhibit a 3-4 fold increase in plasma cortisol as part of the act of initiation of parturition. However subclinically hypocalcemic cows may have 5-7 fold increases in plasma cortisol on the day of calving and the typical milk fever cow may exhibit plasma cortisol concentrations that are 10 –15 fold higher than pre-calving plasma cortisol concentration (Horst and Jorgensen, 1982). Cortisol is generally considered a powerful immune suppressive agent and likely exacerbates the immune suppression normally observed in the periparturient period. Exacerbates rather than causes because most studies suggest that immune suppression begins 1-2 weeks before calving (Kehrli et al., 1989, Ishikawa et al., 1987, Kashiwazaki et al., 1985), and the cortisol surge is fairly tightly confined to the day of calving and perhaps the day after calving.

Kimura et al., (2006), studied calcium fluxes inside peripheral blood mononuclear cells (PBMC) isolated from cows during the transition period. Because intracellular calcium signaling is a key early feature in immune cell activation, we have hypothesized that the increased demand for calcium in periparturient cows may adversely affect intracellular calcium stores of immune cells. This reduction in intracellular calcium stores in immune cells could blunt intracellular calcium release following an activating stimulus, contributing to the immune suppression seen in these animals. To test this hypothesis, peripheral blood mononuclear cells were obtained from 27 multiparous dairy cows spanning a period of 2 wk before and 2 wk after parturition. Following activation of these cells by anti-CD3 antibodies plus secondary antibodies, intracellular calcium release from intracellular stores was measured. The intracellular calcium released in response

to the activation signal declined as calcium demand for lactation became more intense and recovered as plasma calcium normalized. Intracellular calcium stores in peripheral mononuclear cells, estimated by pretreating cells with pervanadate and ionomycin, significantly decreased at parturition and returned to normal levels as the cows' blood calcium returned to normal levels. Hypocalcemia, which is common in periparturient dairy cows, is associated with decreased intracellular calcium stores in peripheral mononuclear cells. Our data suggest that this is the cause of a blunted intracellular calcium release response to an immune cell activation signal. It is concluded that intracellular Ca stores decrease in peripheral blood mononuclear cells (PBMC) before parturition and development of hypocalcemia. This decrease in PBMC intracellular Ca stores before parturition and the development of hypocalcemia contributes to periparturient immune suppression.

KETOSIS AND MASTITIS SUSCEPTIBILITY

Ketosis is diagnosed whenever there are elevated levels of ketones in the blood, urine, or milk of a cow. The disease is always characterized by a decline in blood glucose as well. In lactation, the amount of energy required for maintenance of body tissues and milk production exceeds the amount of energy the cow can obtain from her diet, especially in early lactation when dry matter intake is still low. As a result, the cow must utilize body fat as a source of energy. Every good cow will utilize body reserves in early lactation to help her make milk. However, there is a limit to the amount of fatty acid that can be handled and used for energy by the liver (and to some extent the other tissues of the body). When this limit is reached, the fats are no longer burned for energy but begin to accumulate within the liver cells as triglyceride. Some of the fatty acids are converted to ketones. The appearance of these ketones in the blood, milk, and urine is diagnostic of ketosis. As fat accumulates in the liver it reduces liver function - and a major function of the liver in the dairy cow is to produce glucose.

Research (Grummer, 1993) demonstrates the importance of feed intake at calving on the etiology of the fatty liver-ketosis syndrome. On average, dry matter intake decreases by 20-30% 1 or 2 days before calving, and does not recover until 1 to 2 days after calving (Bertics et al., 1992, Marquardt et al., 1977). Interestingly, liver biopsies showed that liver triglycerides were increased 3-fold by the d of calving. Triglyceride buildup in the liver is a much earlier phenomena than previously assumed. Even more interesting; when cows were fitted with rumen fistulas and dry matter intake was not allowed to drop around the time of calving by forcing feed into the rumen, liver lipids and triglycerides increased only a small amount. Similar results were also achieved by daily drenching of cows with propylene glycol (1 L/d) during the week before and after calving (Studer, et al., 1993). The conclusion is that energy intake must not be compromised during the days around calving. Any factor restricting feed intake around calving (such as milk fever or retained placenta) increases fat accumulation in the liver, affecting the energy deficit of the cow and increasing the risk of fatty liver-ketosis.

The fresh cow is also in negative protein balance shortly after calving. Generally this is not perceived to be as big a problem as the negative energy balance of early lactation but the typical cow will lose 37 lbs body protein during the first 2 weeks of lactation. Much of this body protein is being used to support the amino acid and glucose requirements of milk production (Paquay et al., 1972). Therefore in many respects the dairy cow in early lactation is in a physiological state comparable to that of humans and rodents with prolonged protein-calorie restriction. Glutamine is the most abundant free amino acid in human muscle and plasma and is utilized at high rates by rapidly dividing cells, including leucocytes, to provide energy and optimal conditions for nucleotide biosynthesis. As such, it is considered to be essential for proper immune function. In humans, plasma glutamine is known to fall in patients with untreated diabetes mellitus, in diet-induced metabolic acidosis and in the recovery period following high intensity intermittent exercise (Walsh et al., 1998). Interestingly, plasma glutamine does not seem to be depressed in the dairy cow at calving – when body fat mobilization is rapidly increasing and presumably protein catabolism would also be increasing rapidly. However plasma glutamine does decrease as the cow progresses into the early weeks of lactation (Zhu et al., 2000, Meijer et al., 1995).

Does the relative protein-calorie deficiency of the early lactation cow impact her immune response? In humans, protein-calorie restriction has severe effects on cell–mediated immunity. There is often widespread atrophy of lymphoid tissues and this can cause a 50% decline in the number of circulating T-cells. Surprisingly antibody responses are intact and phagocytosis of bacteria is relatively normal. However destruction of bacteria within the phagocytes is impaired (Roitt, 1991).

A few studies have been done on lymphocytes isolated directly from normal cows and cows with clinical and subclinical ketosis and then placed into culture. Leukocytes of cows with clinical symptoms and the highest concentration of ketones and free fatty acids in blood responded with the lowest levels of interferons alpha and gamma to three interferon inducers: Newcastle Disease Virus, phytohemagglutinin and concanavalin A . Depression in interferon PHA stimulated synthesis correlated with a very low mitogenic response of blood lymphocytes. A correlation between the stage of ketosis and the level of interferon production in milk leukocytes was also observed (Kandefer-Szerszen et al., 1992).

Zerbe et al., (2000) examined the relationship between liver triacyl glycerol content and immunophenotypical and functional properties of neutrophils of dairy cows in the peripartum period. Increased liver TAG content, > 40mg/g which was considered the upper level of normal, went in parallel with a reduced expression of function-associated surface molecules on blood neutrophils. Moreover, in cows with high liver TAG levels the antibody-independent and -dependent cellular cytotoxicity (AICC, ADCC) of blood PMN was markedly reduced. PMN also were less capable of reactive oxygen species generation after stimulation with Phorbol Myristate Acetate.

WHAT IS THE ENERGY COST TO MOUNT AN IMMUNE RESPONSE?

Little to no work has been done to examine this issue in cattle. However, in humans suffering from severe infection causing sepsis (various degrees of fever, increased WBC count, and acute phase protein production), the resting energy expenditure (determined by indirect calorimetry), increased progressively over the first week of the infection to around 40% above normal and was still elevated 3 weeks from the onset of illness. As an aside; over a 3-week period patients lost 13% of their total body protein (Plank and Hill, 2000).

No such measurements have been reported for cattle. However if we are allowed to extrapolate and speculate we can go through a few calculations. Maintenance energy for a 600 kg dairy cow is approximately 9.7 Mcal Net energy / day. If the cow must also increase energy expenditure 40% to mount an inflammatory response the energy requirement increases by nearly 4 Mcal / day. This is roughly equivalent to a requirement that the cow consume an additional 2.4 kg of diet (assuming a diet that provided 1.65 Mcal Nel/kg). Can the periparturient cow, already in negative energy balance, be expected to successfully mount a rapid immune response? If she is in fact in negative protein balance as well, will her immune system produce the immunoglobulins and acute phase proteins necessary to fight an infection while it is still in the acute phase to prevent it from escalating to a clinical infection?

RETAINED PLACENTA

We have recently confirmed studies begun by Gunnink (1984) that suggest the two are likely linked because both are due to immune suppression in affected cows. Gunnink's theory suggested the fetal placenta must be recognized as "foreign" tissue and rejected by the immune system after parturition to cause expulsion of the placenta. We hypothesized that impaired neutrophil function causes retained placenta. We examined the ability of neutrophils to recognize fetal cotyledon tissue as assessed by a chemotaxis assay which utilized a placental homogenate obtained from a spontaneously expelled placenta as the chemoattractant. Neutrophil killing ability was also estimated by determining myeloperoxidase activity in isolated neutrophils. Blood samples were obtained from 142 periparturient dairy cattle in 2 herds. Twenty cattle developed RP (14.1 %). Neutrophils isolated from blood of cows with RP had significantly lower neutrophil function in both assays prior to calving and this impaired function lasted for 1-2 wk after parturition. Addition of antibody directed against interleukin-8 (IL-8) to the cotyledon preparation used as a chemoattractant inhibited chemotaxis by 41% suggesting one of the chemoattractants present in the cotyledon at parturition is IL-8. At calving plasma IL-8 concentration was lower in RP cows (51 ± 12 pg/ml) than in cows expelling the placenta normally (134 ± 11 pg/ml) (Kimura et al., 2002). These data suggest neutrophil function determines whether or not the cow will develop RP. These data also suggest that depressed production of IL-8 may be a factor affecting neutrophil function in cows developing RP. This suppressed immune system could also explain why the same cows are more susceptible to mastitis. Retained

placenta probably does not cause mastitis but is symptomatic of a depressed immune system.

METRITIS AND IMMUNE SUPPRESSION

Upon delivery of a calf the cervical canal remains open for a period of days. This allows fluids and tissue no longer required to maintain pregnancy to leave the uterus, but also permits bacteria to ascend the vagina and gain entry to the uterus. Bacteria can be isolated from uterine culture of most cows during the first week after calving (Hussein et al., 1990; Elliot et al., 1968). In most cows, neutrophils migrate into the lumen of the uterus in response to the initial detection of bacteria. Usually the neutrophils are capable of keeping bacterial populations in check until eventually they, and perhaps other immune cells, can eliminate the bacterial infection. The infection remains low grade or sub-clinical. The cow remains “healthy” and goes on her way toward a productive lactation and subsequent pregnancy. However, in from 15-30% of cows (depending on the study and definition of “infection”) the neutrophils do not successfully halt the infection. In these animals bacterial populations explode and gain the upper hand. Though eventually the immune system will clear these infections (few cows actually die of metritis or endometritis) the inflammatory response required to gain the upper hand once the infection is established is much greater and sustained beyond the periparturient period. In these animals the inflammatory response to these bacterial populations results in a purulent, fetid discharge referred to as metritis. Upon rectal palpation the uterus is enlarged and the inflammation of the uterus suppresses post-partum ovarian growth and follicular development. Around half of cows with metritis will have a fever and most will be depressed and “off feed”. Failure to consume adequate amounts of their ration then predisposes the cow to other disorders such as displaced abomasum and the fatty liver / ketosis complex. Continued inflammation of the uterine lining often progresses to endometritis, which can greatly compromise the fertility of the cow. Estimates suggest a case of metritis costs about $106 / lactation in drug, veterinary services, and lost production costs (Bartlett et al., 1986). If just 15% of the 8.5 million dairy cows in the US develop metritis this year, the disease will cost US dairy producers $135 million.

A number of studies have now demonstrated that cows that will develop metritis and/or endometritis have neutrophils with severely compromised function even before they calve (Kim eta l., 2005; Hammon et al., 2006). Hammon et al (2006) demonstrated that neutrophils of cows that would develop metritis were around 30% as able to generate reactive oxygen species (an index of the ability to kill bacteria) as cells from normal cows. It now seems that it is this severely reduced neutrophil function which predisposes these cows to develop metritis in early lactation. Recently, Martinez et al (2012)demonstrated that sub-clinical hypocalcemia (blood calcium below 8.6 mg/dl) was a major risk factor for metritis. Cows with sub-clinical hypocalcemia were at a greater risk of developing fever, metritis, and puerperal metritis compared with normocalcemic cows. Cows with sub-clinical hypocalcemia had elevated concentrations of nonesterified fatty acids and β-hydroxybutyrate compared with normocalcemic cows. The relative risk of developing metritis decreased by 22% for every 1mg/dL increase in serum Ca. Cows

with sub-clinical hypocalcemia had a reduced pregnancy rate and a longer interval to pregnancy compared with normocalcemic cows.

In addition reduced neutrophil function appears to be a pre-disposing factor contributing to the risk of retained fetal membranes (Kimura et al., 2002; Gunnink, 1984). These studies do not demonstrate cause and effect for retained fetal membranes but do suggest that the functioning neutrophil plays a role in separation of maternal and fetal tissues at the time of calving. Unfortunately, despite numerous efforts to improve neutrophil function in cows around the time of calving there has been little success.

PREVENTING HYPOCALCEMIA

Our lab and a few others now believe the key to milk fever prevention lies in reducing the degree of metabolic alkalosis typical of a cow fed a high potassium diet. As summarized in Goff et al, (2008) the Current hypothesis revolves around parathyroid hormone (PTH) effects at the surface of target bone and kidney cells under various physiological circumstances. Under normal conditions, PTH released in response to hypocalcemia interacts with its receptor, located on the surface of bone and kidney cells, in a lock and key fashion. This stimulates G- coupled receptor proteins and the enzyme adenylate cyclase (adenylate cyclase complex) resulting in production of cyclic AMP, which acts as a second messenger within the cytosol of target cells. This initiates mechanisms such as bone Ca resorption and renal production of 1,25-dihydroxyvitamin D to restore blood Ca concentration to normal levels. Alkalotic conditions induced by high potassium diets induce a change in the shape of the PTH receptor protein so that it is less able to recognize and bind PTH, resulting in failure to activate the cell by producing cyclic AMP. The bone and kidney cells do not respond and the cow gets milk fever. Mg is also required for full function of the adenylate cyclase complex. Hypomagnesemia reduces ability of PTH stimulated cells to produce cyclic AMP, resulting in failure to activate the cell, again resulting in hypomagnesemia.

To ensure adequate concentrations of Mg in the blood of the periparturient cow the dietary Mg concentration should be 3.5–4.0 g/kg (0.35–0.4%). This higher dietary Mg concentration allows the cow to take advantage of passive absorption of Mg across the rumen wall. Dietary P concentration should be fed at a level to meet the NRC requirement for P in the late gestation cow. This is generally about 4 g/kg (0.4%) P for most cows. A diet supplying more than 80 g P/day (Barton, 1978; Kichura et al., 1982) will block renal production of 1,25 dihydroxyvitamin D and will actually cause milk fever. Dietary S must be kept above 0.22% to ensure adequate substrate for rumen microbial amino acid synthesis. Corn (maize) silage diets are notoriously low in sulfur. Now, with the exception of K and Cl, the ‘variables’ in the various proposed DCAD equations have become more or less ‘fixed’. The key to milk fever prevention (at least with Holstein cows) is to keep K as close to the NRC requirement of the dry cow as possible (about 10 g/kg or 1.0% diet K). The key to reduction of subclinical hypocalcemia, not just milk fever, is to add Cl to the ration to counteract the effects of even low diet K on blood alkalinity. For formulation purposes, the concentration of Cl required in the diet to acidify

the cow is approximately 5 g/kg (0.5%) less than the concentration of K in the diet. In other words, if diet K can be reduced to 13 g/kg (1.3%), the Cl concentration of the diet should be increased to 8 g/kg (0.8%). Add Cl at this level and observe urine pH after 3–4 days. This is often a conservative approach and the final concentration of Cl needed to truly acidify the urine may have to be brought up to within 4 or even 3 g/kg (0.4–0.3%) of dietary K. It is important never to start an anion supplement program with higher levels of Cl. If cows are over-acidified at the onset it becomes very difficult to evaluate urine pH as feed intake will quickly be affected. The dry cow pen should always be worked up to the adequate Cl dose. If dietary K can not be reduced below 20 g/kg (2.0%) the diet Cl would need to be at least 15 g/kg (1.5%) to acidify the cow. This level of Cl in the diet is likely to cause a decrease in dry matter intake independent of over-acidification. Chloride sources differ in their palatability and since achieving low dietary K can be difficult it is prudent to use a palatable source of Cl when formulating the diet. Ammonium chloride (or ammonium sulfate) can be particularly unpalatable when included in rations with a high pH. At higher pH, a portion of the ammonium cation is converted to ammonia, which is highly irritating when smelled by the cow.

REFERENCES

Bartlett, P.C.; J.H. Kirk, M.A. Wilke, J.B. Kaneene and E.C. Mather , Metritis complex in Michigan Holstein–Friesien cattle: incidence, descriptive epidemiology and estimated economic impact. Prev. Vet. Med. 4 (1986), pp. 235–248.

Barton, B.A., 1978. Studies of vitamin D, calcium, and phosphorus metabolism of the dairy cow. Master's thesis dissertation. University of Wisconsin, Madison, WI, USA.

Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, D. W. LaCount, E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early postpartum lactation. J. Dairy Sci. 75:1914.

Boulay M, Scott ME, Conly SL, Stevenson MM, Koski KG. 1998. Dietary protein and zinc restrictions independently modify a Heligmosomoides polygyrus (Nematoda) infection in mice. Parasitology 116 (Pt 5):449-62

Christadoss P, Talal N, Lindstrom J, Fernandes G. 1984. Suppression of cellular and humoral immunity to T-dependent antigens by calorie restriction. Cell Immunol. 88(1):1-8.

Daniel, R. C. W. 1983. Motility of the rumen and abomasum during hypocalcaemia. Can. J. Comp. Med. 47:276.

Doherty ML, Monaghan ML, Bassett HF, Quinn PJ, Davis WC. 1996. Effect of dietary restriction on cell-mediated immune responses in cattle infected with Mycobacterium bovis. Vet Immunol Immunopathol 49(4):307-20

Elliot, L. K.J. McMahon, H.T. Gier and G.B. Marion, Uterus of the cow after parturition: bacterial content, Am J Vet Res 29 (1968), pp. 77–81.

Franklin ST, Young JW, Nonnecke BJ. 1991. Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. J Dairy Sci 1991 74:2507-14

Goff JP, Horst RL, Jardon PW, Borelli C, Wedam J. 1996. Field trials of an oral calcium propionate paste as an aid to prevent milk fever in periparturient dairy cows. J Dairy Sci. 79:378-83.

Goff JP. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet J. 2008 Apr;176(1):50-7.

Gregory R, McElveen J, Tattersall RB, Todd I. 1993. The effects of 3-hydroxybutyrate and glucose on human T cell responses to Candida albicans. FEMS Immunol Med Microbiol 7:315-20

Grummer, R. R. 1993. Etiology of lipid related disorders in periparturient dairy cows. J. Dairy Sci. 76:3882.

Gunnink, J. W. 1984. Pre-partum leucotytic activity and retained placenta. Vet. Q. 6: 52-54.

Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immunol Immunopathol. 2006 15;113(1-2):21-9.

Hoeben D, Heyneman R, Burvenich C. 1997. Elevated levels of beta-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils. Vet Immunol Immunopathol. 58:165-70

Horst, R. L., and N. A. Jorgensen. 1982. Elevated plasma cortisol during induced and spontaneous hypocalcemia in ruminants. J. Dairy Sci. 65:2332.

Hussain, A.M.; R.C.W. Daniel and D. O'Boyle, Postpartum uterine flora following normal and abnormal puerperium in cows, Theriogenology 34 (1990), pp. 291–302.
Ishikawa, H. 1987. Observation of lymphocyte function in perinatal cows and neonatal calves. Jpn. J. Vet. Sci. 49:469.
Kandefer-Szerszen M, Filar J, Szuster-Ciesielska A, Rzeski W. 1992. Suppression of interferon response of bovine leukocytes during clinical and subclinical ketosis in lactating cows. Dtsch Tierarztl Wochenschr 99:440
Kashiwazaki, Y., Y. Maede, and S. Namioka. 1985. Transformation of bovine peripheral blood lymphocytes in the perinatal period. Jpn. J. Vet. Sci. 47:337.
Kehrli, Jr., M. E., B. J. Nonnecke, and J. A. Roth. 1989a. Alterations in bovine neutrophil function during the periparturient period. Am. J. Vet. Res. 50:207.
Kehrli, M. E. Jr, B. J. Nonnecke, and J. A. Roth. 1989b. Alterations in bovine lymphocyte function during the periparturient period. Am. J. Vet. Res. 50:215.
Kehrli ME Jr, Goff JP. 1989. Periparturient hypocalcemia in cows: effects on peripheral blood neutrophil and lymphocyte function. J Dairy Sci. 72:1188-96.
Kehrli ME Jr, Goff JP, Harp JA, Thurston JR, Norcross NL. 1990. Effects of preventing periparturient hypocalcemia in cows by parathyroid hormone administration on hematology, conglutinin, immunoglobulin, and shedding of Staphylococcus aureus in milk. J Dairy Sci. 73(8):2103-11.
Kehrli ME Jr, Shuster DE. 1994. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. J Dairy Sci. 77(2):619-27.
Kichura, T.S., Horst, R.L., Beitz, D.C., Littledike, E.T., 1982. Relationships between prepartal dietary calcium and phosphorus, vitamin D metabolism and parturient paresis in dairy cows. The Journal of Nutrition 112, 480–487.
Kim IH, Na KJ, Yang MP. Immune responses during the peripartum period in dairy cows with postpartum endometritis. J Reprod Dev. 2005 51(6):757-64.
Kimura K, Goff JP, Kehrli ME Jr. 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. J Dairy Sci. 82:2385-92.
Kimura K, Goff JP, Kehrli ME Jr, Reinhardt TA. Decreased neutrophil function as a cause of retained placenta in dairy cattle. J Dairy Sci. 2002. 85(3):544-50
Kimura K, Reinhardt TA, Goff JP. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J Dairy Sci. 2006 Jul;89(7):2588-95.
Leiba A, Amital H, Gershwin ME, Shoenfeld Y. 2001. Diet and lupus. Lupus 10:246-8.
Marquardt, J. P., R. L. Horst, and N. A. Jorgensen. 1977. Effect of parity on dry matter intake at parturition in dairy cattle. J. Dairy Sci. 60:929.
Martinez N, Risco CA, Lima FS, Bisinotto RS, Greco LF, Ribeiro ES, Maunsell F, Galvão K, Santos JE. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J Dairy Sci. 2012 95(12):7158-72.
Meijer GA, Van der Meulen J, Bakker JG, Van der Koelen CJ, Van Vuuren AM. 1995. Free amino acids in plasma and muscle of high yielding dairy cows in early lactation. J Dairy Sci. 78:1131-41.
Nonnecke BJ, Franklin ST, Young JW. 1992. Effects of ketones, acetate, and glucose on in vitro immunoglobulin secretion by bovine lymphocytes. J Dairy Sci 75:982-90.

Paquay R, de Baere R, Lousse A. 1972. The insensible loss of weight in dairy cows. Z Tierphysiol Tierernahr Futtermittelkd. 30(4):202-7.
Plank LD, Hill GL. 2000. Sequential metabolic changes following induction of systemic inflammatory response in patients with severe sepsis or major blunt trauma. World J Surg 24:630-8
Roitt, I. M. 1991. Essential Immunology, Seventh edition. Blackwell Scientific Publications, Oxford, England. P. 170.
Sartorelli P, Paltrinieri S, Comazzi S. 2000. Non-specific immunity and ketone bodies. II: In vitro studies on adherence and superoxide anion production in ovine neutrophils. J Vet Med A Physiol Pathol Clin Med. 47:1-8
Stapleton PP, Fujita J, Murphy EM, Naama HA, Daly JM. 2001. The influence of restricted calorie intake on peritoneal macrophage function. Nutrition. 17:41-5.
Studer, V. A., R. R. Grummer, S. J. Bertics, C. K. Reynolds. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. J. Dairy Sci. 76:2931
Swierenga SH, MacManus JP, Braceland BM, Youdale T. 1976. Regulation of the primary immune response in vivo by parathyroid hormone. J Immunol 117(5 Pt 1):1608-11.
Walsh NP, Blannin AK, Robson PJ, Gleeson M. 1998. Glutamine, exercise and immune function. Links and possible mechanisms.
Sports Med 26:177-191
Zerbe H, Schneider N, Leibold W, Wensing T, Kruip TA, Schuberth HJ. 2000. Altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. Theriogenology 54:771-86
Zhu LH, Armentano LE, Bremmer DR, Grummer RR, Bertics SJ. 2000.
Plasma concentration of urea, ammonia, glutamine around calving, and the relation of hepatic triglyceride, to plasma ammonia removal and blood acid-base balance. J Dairy Sci 83:734-40