THE INFLUENCE OF NEGATIVE ENERGY BALANCE ON UDDER HEALTH

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Introduction

The immense change in requirement for energy by the dairy cow at the time of calving is well documented (3, 17). This increased requirement occurs at a time when dry matter intake is reduced. The homeorhetic drive to sustain high levels of milk production necessitates mobilization of body fat stores, and results in elevated levels of circulating ketone bodies in early lactation. Ketone bodies are intermediate metabolic products. They provide available energy to peripheral tissues when carbohydrate levels are limited. Furthermore, betahydroxybutyrate (BHB) is also utilized for milk fat synthesis (28). Therefore, a baseline level of circulating ketone bodies (BHB, acetoacetate and acetone) is normal in the postparturient dairy cow. However, as the levels of ketones continue to increase, and are sustained at an elevated level, an abnormal state known as ketosis occurs.

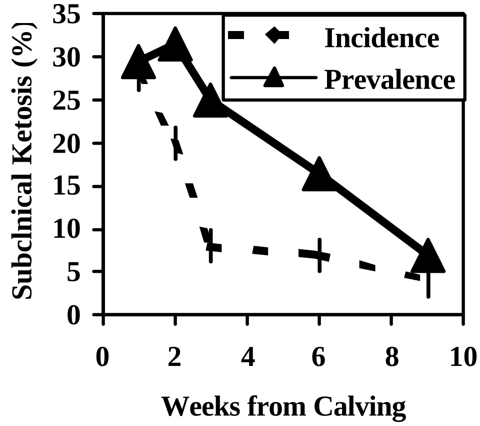
Subclinical ketosis in dairy cattle results from a prolonged negative energy balance and is characterized by the presence of an excess level of circulating ketone bodies in the absence of clinical signs of ketosis (1). Negative energy balance and subclinical ketosis have been extensively reviewed (2, 3, 22, 23, 10). In negative energy balance, the elevated ketone bodies are present in blood, urine and milk (4). Both cowside and laboratory tests are available to measure elevated ketone bodies in these fluids (10). However, the changes in levels of the various ketones over time, and the impact on these changes on animal productivity and test performance are not well defined.

The purpose of this paper is to briefly describe what is known about the prevalence and impact of subclinical ketosis on dairy cattle. The association between negative energy balance and intramammary infection will be reviewed. Results of studies on the outcome of experimentally induced mastitis relative to energy balance will be described. Field studies on the associations between naturally occurring subclinical ketosis and measures of impaired udder health will also be presented.

Subclinical Ketosis

Reports on the incidence and prevalence of either clinical or subclinical ketosis vary widely based upon the population at risk, test methods and the cut-off levels used for ketones in blood or milk (10). However, recent studies conclude that approximately 50% of all lactating cows go through a stage of subclinical ketosis in early lactation (10, 12). Canadian research has reported that the cumulative incidence of subclinical ketosis in the first nine weeks of lactation was 59% and 43% using cut-off thresholds of ≥1200 and ≥1400 ìmol/L BHB in serum, respectively. The peak incidence occurred in the first week postpartum (Figure 1). An estimate of the average duration of subclinical ketosis is about 16 days (6).

Figure 1. Incidence and Prevalence of Subclinical Ketosis In Lactating Dairy Cows. (Data from the placebo group of a large clinical trial involving 1010 Holstein Dairy Cows and 25 dairy farms). From Duffield, 2000 (10).



Important risk factors for subclinical ketosis have been reviewed (10). The predominant factors identified include herd, parity, body condition, genetic predisposition and season. Large differences have been shown in the prevalence and incidence of subclinical ketosis between herds. Feeding frequency and the level of concentrates fed are two of the probable reasons for these differences. Many researchers report a higher prevalence of hyperketonemia with increasing lactation number (6, 10). Body condition score (BCS) prior to calving is an important risk factor for subsequent development of subclinical ketosis during lactation (13, 19). Cows at BCS $\geq$ 4.0 were at highest risk, and had the highest BHB concentrations, compared to normal and thin cows prior to calving. Genetic predisposition and breed differences account for some of the variation in subclinical ketosis rates. Seasonal differences also occur, and depend upon geographic region. In North America, subclinical ketosis rates may be highest in the summer due to suppressed feed intake, changes in forages, and reduced management intensity.

Subclinical Ketosis and Periparturient Disease

The association between subclinical or clinical ketosis and the other common periparturient disease has recently been reviewed (10). Several epidemiological studies have described these associations; usually concluding that it is a complex situation, and difficult to make causal inferences. There is an association between subclinical ketosis and metritis (6). Most often, the subclinical ketosis is identified first, but this relationship is debated. Similar discussions have occurred relative to abomasal displacement. However, recent research has clarified this situation. The likelihood of abomasal displacement was significantly increased when BHB concentrations were elevated above 1400 ìmol/L (14). In addition, cows with BHB at or above 1400 ìmol/L in the first two weeks post-calving were three times more likely to develop abomasal displacement (9). The associations between subclinical ketosis and mastitis will be described in the subsequent sections of this paper.

Hyperketonemia and the Impairment of Udder Defence

A review of the published literature on aspects of energy balance and udder defence has recently been completed and published (30). Cows in the negative energy balance period show an impairment of udder defence mechanisms. Hyperketonemia is hypothesized as one of the most important factors leading to reduced udder defences. There are possible explanations for these effects via each of the mechanisms of defence.

Firstly, the capacity for phagocytosis by polymorphonuclear cells and macrophages may be reduced in negative energy balance. In cows without intramammary infection (IMI), SCC is positively correlated with BCS. Perhaps in cows losing weight, SCC is lowered. It is also clear that bacterial killing capacity is impaired in the presence of ketone bodies. It is not yet conclusive that ketone bodies reduce the capacity for phagocytosis. Secondly, there is the capacity for udder leukocytes to induce cell recruitment in IMI. This may be due to lower amounts of cytokines produced by lymphocytes in ketotic cows. These cytokines include interferon, interleukins and tumour necrosis factor. Overall, it is likely that the generation of chemoattractant is reduced in hyperketonemic cows. Finally, the capacity for blood leukocytes to migrate into the infected gland is reduced. Leukocyte chemotaxis has been shown to be significantly reduced in a ketone body environment. From the review of this subject area by Suriyasathaporn et al., 2000 (30), as described in Table 1, it is clear that leukocytes from hyperketonemic cows have impaired udder defence mechanisms against mastitis.

Table 1. Summary of ketosis affecting an acute phase of udder defense mechanism

		Functional Capacity	
System	Item	Leukocytes from ketotic subjects	Leukocytes in media supplemented with ketone
Phagocytic defence			
	Phagocytosis	Unchanged in ketotic humans	Inhibit PMN phagocytosis
	Bactericidal activity	Amount of superoxide anion produced by leukocytes was lower in NEB humans	Suppression when cultured in the presence of BHB
Release of inflammatory mediator			
	Opsonin	Lower IgG level after tetanus toxoid immunization	-----------
	Chemoattractants	Amount of cytokines produced by leukocytes was lower in ketotic than in normal cows	Cytokines produced by leukocytes decreased when cultured in whole blood from NEB human
Leukocytes chemotaxis			
	Number of blood leukocytes	Lower in relation to BHB level in ketotic cows	Ketone added in culture media inhibited the proliferation of bovine bone marrow cells
	Chemotaxis	Lower when leukocytes obtained from spontaneous ketotic cows	Lower when cultured in the presence of ketone bodies

From Suriyasathaporn, 2000. (30)

Metabolic Status and Outcome of Experimentally Induced Mastitis

At least two well-designed studies have been reported on the severity of experimentally-induced mastitis in ketotic cows (21, 31). In the first study, the severity of experimental *E. coli* mastitis was investigated in cows during a period of negative energy balance (21). The state of negative energy balance was created by feed restriction. After the restriction process, cows were classified as ketonemic or non-ketonemic based on the BHB concentration in blood. *E. coli* growth in the milk from inoculated quarters was used as a parameter for the severity of the experimental mastitis. In non-ketotic cows, the experimental mastitis was moderate to severe. The severity was negatively related to preinfection chemotactic response. In contrast, the course of experimental mastitis was relatively severe in all cows, regardless of the preinfection chemotactic response (21).

In a follow-up experiment, the effect of metabolic status of cows around calving on the severity of an experimentally induced IMI was investigated. Seven cows were intensively followed for metabolic indicators in blood from two weeks prepartum to three weeks postpartum. Each cow was challenged with *E. coli* in the left rear quarter. Concentration of BHB showed a strong positive correlation to the severity of mastitis. Glucose, NEFA and cholesterol were also related to BHB. Several host response measures were significantly related to the severity of infection, including the concentration of circulating leukocytes, the magnitude of leukocyte influx into the gland and the cortisol response after infection (31).

The cited experimental mastitis studies allow us to conclude that negative energy balance and ketonemia are related to the severity of induced *E. coli* mastitis. The exact mechanisms involved remain unclear.

Metabolic Status and Measures of Udder Health

Several epidemiological studies have shown that clinical ketosis is associated with an increased risk of clinical mastitis (5, 11, 18, 27). Recently, investigations of negative energy balance in a substantial population of dairy cows have been analyzed for interactions between subclinical ketosis and measures of udder health.

In a study to evaluate the effect of monensin on the incidence of subclinical ketosis, approximately 1000 cows were blood samples at 1, 2, 3, 6, and 9 weeks after calving (7). The associations between subclinical ketosis and clinical mastitis are shown in Table 2. Overall, 15.1% (39 of 258) of cows with subclinical ketosis at any week had clinical mastitis, as compared to 10.1% (70 of 693) non-ketotic cows. This difference was significant ($p \leq 0.05$). The magnitude of this association was greatest in cows with ketonemia during the first week postpartum, when 18.1% of ketotic cows had clinical mastitis ($p \leq 0.01$). In order to evaluate these associations, while controlling for other factors known to affect clinical mastitis rates, multi-variable regression analysis was conducted. It was found that parity, calving in summer and fall seasons, and being ketonemic at a threshold of ≥1400 ìmol/L BHB was significantly associated with an increased risk of clinical mastitis.

Table 2. Associations between Subclinical Ketosis (³ 1400 ìmol/L BHB) and Clinical Mastitis in 951 Ontario Dairy Cows.

Week of Lactation	% of Ketotic Cows with Mastitis	% of Non-ketotic Cows with Mastitis	Significance Level
Week 1	18.1 (29/160)	10.1 (80/791)	$p \leq 0.01$
Week 2	14.6 (26/178)	10.7 (83/773)	$p = 0.14$
Overall	15.1 (39/258)	10.1 (70/693)	$p \leq 0.05$

The associations between subclinical ketosis and elevated SCC in this population are shown in Table 3. Of cows with ≥1400 ìmol/L BHB, 29.4% had an elevated SCC as compared to 21.4% in the non-ketotic cows ($p \leq 0.05$). Of cows with ketonemia for two or more weeks in the postpartum period, 21.6% had an elevated SCC, as compared to 13.6% of cows without chronic ketonemia ($p \leq 0.01$). A logistic regression model was used to evaluate the risk of elevated SCC in cows with chronic ketonemia. Controlling for all other factors, chronically ketonemic cows were 1.7 times more likely to have an elevated SCC than cows that were not ketonemic for two or more weeks ($p \leq 0.05$).

Table 3. Associations between Subclinical Ketosis (³ 1400 ìmol/L BHB) and Elevated SCC in Ontario Dairy Cows.

Weeks Positive for Ketosis	% of Ketotic Cows with Elevated SCC	% of Non-ketotic Cows with Elevated SCC	Significance Level
Once in any week of 1, 2, 3, 6 or 9	29.4 (59/197)	21.4 (76/355)	$p \leq 0.05$
Two or more weeks positive	21.6 (31/134)	13.6 (57/418)	$p \leq 0.01$

In another population of 1142 cows in 20 herds, blood samples were collected in the week before calving and in the first week postpartum (24). Mastitis was monitored as a producer diagnosis of clinical signs in the first 30 days in milk. There was a 9.7% lactational incidence rate (111 cases) using this definition. Of cows with serum BHB ≥1400 ìmol/L in the week before calving, 28.6% subsequently had clinical mastitis as compared with 8.7% of non-ketonemic prepartum cows ($p \leq 0.05$). Further analyses of these data are in progress (24). Of particular interest is an apparent markedly elevated serum glucose level on the day of calving in cows subsequently treated for clinical mastitis in the early postpartum period.

Prevention of Ketosis and its Impact on Mastitis

Recommendations for the prevention of subclincial ketosis have focused on the nutritional management of the dry and transition cow. This subject area has been reviewed (26). Of particular note is the reduction of overconditioning in late lactation and dry cows, as well as prepartum lead feeding of concentrates (1, 22). In addition to nutritional recommendations, prophylactic administration of certain feed additives have been found to be beneficial in reducing subclinical ketosis. These additives include niacin, propylene glycol, sodium propionate, and ionophores (10). Of these additives, ionophores are relatively inexpensive and much easier to administer, as well as having excellent prophylactic potential for reducing hyperketonemia. Several publications have documented the effect of monensin on peripartum metabolic parameters (10). Monensin has also been shown to improve the chemotactic function of bovine neutrophils (29). A controlled release capsule that delivers 335 mg of monensin sodium per day for 95 days reduced the incidence of subclinical ketosis by 50% when it was administered three weeks prior to expected calving (8). This product has gained regulatory approval, and widespread use, in several countries (i.e. Canada and Australia). There is potential for a major impact on the prevention of periparturient disease through alleviation of negative energy balance with the prophylactic use of nutritional additives, such as monensin.

In recently reported research, twelve trials designed to test the effect of monensin on milk production carried out at eight research farms were evaluated for effects of monensin on health and reproduction (20). Monensin was added to the concentrate starting either two week before (293 cows) or five weeks after calving (601 cows) for period ranging 16-37 weeks. Applied after calving, the incidence of clinical mastitis was reduced in the monensin treated animals. Monensin decreased the rate of intramammary infection (approximated by a change from below to above 250,000 somatic cells in milk) in first lactation heifers by 13%. No significant monensin effect was observed for the duration of intramammary infection (somatic cells in milk above 250,000). This report is further evidence of the prophylactic effect of monensin on negative energy balance, and its associated negative impact on udder health.

The other aspect of a preventive program is the implementation of monitoring efforts for subclinical ketosis. Serum profiles show promise when limited to specific parameters and targeted at critical time period. However, they remain as somewhat costly and cumbersome laboratory tests. Several cowside tests for ketones in milk have been evaluated (15, 16). Some of these cowside tests have sufficient sensitivity and specificity to be useful monitors for negative energy balance. However, further research on programs to detect negative energy balance at its earliest occurrence is needed.

Summary and Conclusions

Subclincial ketosis is an important metabolic disease in early lactation dairy cattle. Since the etiology is related to the homeorhetic drive for high levels of milk production, the incidence of this condition is likely to remain important. Subclinical ketosis is highly associated with several periparturient diseases, including subclinical and clinical mastitis. There is growing evidence that the mechanisms of udder defence against mastitis are impaired in periods of negative energy balance and hyperketonemia. The results of experimentally induced mastitis in ketotic versus non-ketotic cows have elucidated some of the reasons for the negative impact of subclinical ketosis. There is also mounting evidence from observational studies and clinical trials that increased rates of mastitis associated with subclinical ketosis. Nutritional management and monitoring program show promise for alleviation of the impact of negative energy balance on mastitis and other periparutrient diseases.

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