Severity of Experimental *Escherichia coli* Mastitis in Ketonemic and Nonketonemic Dairy Cows

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ABSTRACT

The severity of experimental *Escherichia coli* mastitis in relation to in vitro chemotaxis of polymorphonuclear leukocytes was investigated in cows during negative energy balance. The negative energy balance was induced by feed restriction. Cows were classified into two groups, ketonemic and nonketonemic, based on the β-hydroxybutyrate concentration in the peripheral blood at the moment of inoculation. Bacterial growth in the inoculated quarter was used as a parameter to indicate the severity of experimental mastitis. In the nonketonemic cows, experimental mastitis ranged from moderate to severe. Severity of experimental mastitis was negatively related to preinfection chemotactic response of polymorphonuclear leukocytes. In contrast, the course of experimental mastitis in the ketonemic group was relatively severe in all cows, regardless of preinfection chemotactic response.
(**Key words**: ketosis, polymorphonuclear leukocytes, *Escherichia coli* mastitis, chemotactic response)

Abbreviation key: **AUC** = area under the curve, **PMNL** = polymorphonuclear leukocytes, **WBC** = white blood cells.

INTRODUCTION

Clinical signs of *Escherichia coli* mastitis in dairy cows show great variability among cows. In particular, high producing cows may be severely diseased during the early postpartum period (7, 15). The primary cause of the variability of severity of *E. coli* mastitis in these cows is unclear. The functional activity of polymorphonuclear leukocytes (**PMNL**), in particular migration of PMNL from blood into the gland cistern, has been considered to be important in determining the severity of *E. coli* mastitis (3, 12, 14, 15, 18, 25).

Epidemiologic studies indicate an association between negative energy balance during early lactation and increased susceptibility to infectious diseases such as mastitis (8, 11). In an in vivo study, experimentally induced, ketonemic calves were shown to be more susceptible to respiratory tract infections than were healthy calves (28). Negative energy balance may lead to increased fat mobilization and increased hepatic ketogenesis (1). Increased concentration of BHBA to >1.4 mmol/L is an indicator of a negative energy balance (1, 4). The physiological mechanisms by which negative energy balance or ketosis lead to increased susceptibility to infectious diseases are poorly understood.

The relationship between ketones or glucose and immune cell function has been investigated (10, 16, 17, 24, 27). One study described an inhibitory effect of ketone bodies on the in vitro phagocytic activity of blood and milk macrophages and PMNL (16). In vitro oxidative metabolism of human PMNL was

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suppressed by BHBA concentrations that were comparable with blood BHBA concentration during ketoacidosis in diabetic patients (27). In vitro function of bovine lymphocytes may be inhibited (17) by ketone concentrations associated with clinical ketosis. Concentrations of acetate associated with ketosis suppressed lymphocyte proliferation in vitro (10). Glucose and ketone plasma concentrations associated with ketosis did not alter in vitro IgM secretion by lymphocytes (24) or lymphocyte proliferation in vitro (10).

In the present study, the severity of experimental *E. coli* mastitis was investigated in ketonemic and nonketonemic cows. Investigated also was the relationship between preinfection PMNL chemotactic response and severity of experimental *E. coli* mastitis with ketonemic status of dairy cows.

MATERIALS AND METHODS

Cows

Eighteen clinically healthy dairy cows of the Dutch Friesian breed or crossbreeds (Dutch Friesian × Holstein Friesian) in their third to sixth lactation and at wk 3 to 6 of lactation were used. All cows had calved normally and showed no clinical signs of periparturient diseases. Foremilk samples from inoculated (rear right) and control (rear left) quarters were bacteriologically negative for major pathogens and had SCC <250,000 cell/ml. Cows were purchased from regular dairy farms and transported to the research institute 1 wk before induction of experimental mastitis. Cows were housed in a tie-stall barn. Milkings were at 0100 and 1400 h.

Experimental Procedure

Preliminary studies in our lab indicated that ration of wilted grass silage fed at approximately half of ad libitum intake, plus only 1 kg/d of concentrate, resulted in increased peripheral blood BHBA concentrations after approximately 4 d.

To induce ketonemia at the time of induction of experimental *E. coli* mastitis in 6 cows, feed restriction was started 4 d before the induction. Twelve more cows, designated unrestricted, were fed wilted grass silage for ad libitum intake and 8 kg/d of concentrates. Water was provided for ad libitum intake to both groups during the entire experimental period. After inoculation, wilted grass silage and concentrates were supplied for ad libitum intake to both groups. Experimental mastitis was induced as previously described (18). Briefly, the right rear quarters of all cows were aseptically infused with 1000 cfu *E. coli* in 20 ml of saline (50 cfu/ml) at 0700 h. An encapsulated strain of *E. coli* 0:157 isolated from a cow with clinical mastitis (21) was used in these experiments. Infused quarters were not milked until the second milking after inoculation (1400 h). Rectal temperatures and heart rate were determined twice daily during a 5-d period before inoculation. From 4 h preinoculation to 12 h postinoculation, rectal temperature and heart rate were recorded every 30 min. From 12 to 36 h postinoculation, rectal temperature and heart rate were determined hourly. From 36 h to 6 d postinoculation, rectal temperature and heart rate were determined three times daily (18). To assess bacterial growth in the inoculated quarters, the number of *E. coli* in foremilk samples collected aseptically at 8, 15, 22, 32, 46, 54, 72, 104, and 120 h postinoculation was determined. Bacterial counts were made using a spiral-plater (Lameris Laboratory, Breukelen, The Netherlands) on violet red bile glucose agar (CM 484; Oxoid, Hampshire, England).

Bacterial growth in the inoculated quarters was used to determine the severity of the disease (13, 14, 18, 21). For leukocyte differential counts, jugular vein puncture blood samples were collected in vacutainer tubes (Venoject®; Terumo Corp., Tokyo, Japan), with heparin as anticoagulant, at 48 h, 24 h, and immediately prior to inoculation. Concentrations of BHA and glucose were determined in serum samples obtained at d 4, 3, 2, and 1; at 1 h preinoculation; and immediately prior to inoculation. Concentrations of BHBA and glucose were estimated with timed end point method (30°C) with BHBA dehydrogenase as enzyme and NAD as substrate. Glucose concentrations were also estimated with a time end point method (30°C) using hexokinase and glucose-6-phosphate dehydrogenase as enzymes and ATP and NAD as substrates. The procedures were done on a Synchron CX5 (Beckman, Mijdrecht, The Netherlands).

Chemotaxis Assay

Jugular vein blood samples for isolation of cells for chemotaxis assay were collected at 48 h and 24 h preinoculation and immediately prior to inoculation. Blood samples were obtained in polypropylene tubes (19) containing 1 ml of sodium tricitrate (3.8%) solution as anticoagulant. In vitro PMNL chemotactic response was determined in white blood cell (WBC) suspensions (18) using the under agarose assay (23). Pooled bovine serum, obtained from 10 normal healthy cows, was used as chemoattractant. The same batch of pooled serum was used in the experiment.

Migration distance was measured using an ocular micrometer in a stereo microscope at 25× magnification. The chemotactic response was expressed as the chemotactic differential and the chemotactic index. The chemotactic differential was defined as the difference between directed and random migration of PMNL, and the chemotactic index was defined as directed migration divided by random migration (23). Results were the mean of five observations per sample.

WBC Isolation

White blood cells were isolated from the peripheral blood as described previously (19). Briefly, blood samples were centrifuged for 20 min at 1000 × *g*. The plasma layer was discarded for isolation of WBC. The plasma and the buffy coat layers were discarded for isolation of purified PMNL. Erythrocytes were lysed in two steps by hypotonic lysis. After the cells had been washed twice in Eagle's Minimal Essential Medium (Flow Laboratories, Irvine, England), they were resuspended in Eagle's Minimal Essential Medium and adjusted to 5×10^7 cells/ml by an automatic cell counter (Sysmex K-1000; Goffin, IJsselstein, The Netherlands).

Statistical Analyses

Rectal temperature and heart rate were expressed as differences from preinfection baseline values. Baselines were defined as the mean rectal temperature and heart rate during the last 24 h before inoculation. Total daily milk production and milk production in infected and control quarters were expressed as percentages of the preinfection baseline determined for each variable and cow. The baselines for milk production were defined as the mean milk production during the last 4 d before inoculation. Baseline characteristics of nonketonemic and ketonemic cows were compared by using a two-sample *t* test.

To express bacterial growth in infected right rear quarters, the area under the curve (AUC) ($\log_{10}$ *E. coli* bacterial count time) was calculated for the first 5 d postinoculation. The AUC was calculated for each cow as

$$AUC = [(t_i - t_{i-1})f_{i-1}] + [.5(t_i - t_{i-1})(f_i - f_{i-1})]$$

where

t_i = time of observation,
t_{i-1} = previous time of observation,
f_i = $\log_{10}$ bacterial number at time i, and
f_{i-1} = $\log_{10}$ bacterial number at time t i − 1.

Means, standard deviations, linear regressions, correlations, and two sample *t* tests were computed using a microcomputer package (Statistix® NH Analytical Software; Roseville, MN).

Linear regression was used to evaluate the relationship between chemotactic response at 48 h, 24 h, and just before inoculation and the severity of the experimental mastitis with respect to the BHBA concentrations in the peripheral blood. The BHBA concentration in the peripheral blood was a continuous variable in the regression model. For a graphic presentation of the relationship between chemotactic response of PMNL in WBC suspensions and the severity of the experimental mastitis, cows were classified as ketonemic (energy deficient) or nonketonemic. As cutoff point, a BHBA concentration of 1.4 mmol/L was used.
The following linear regression model was used:

$$Y_i = a + b_1CR_i + b_2K_i + b_3(CR \times K) + e$$

where

Y = AUC of the cow i,
a = intercept,

CR_i = preinfection chemotactic response of the cow i,
K_i = BHBA concentration just before inoculation, or
= 1 when BHBA >1.4 mmol/L and 0 when BHBA <1.4 mmol/L,
$CR \times K$ = interaction between preinfection chemotactic response and K_i,
b_1, b_2, b_2 = regression coefficients, and
e = error term.

RESULTS

Preinfection Parameters

Concentrations of BHBA, glucose concentrations in the peripheral blood, AUC, and classification of cows as nonketonemic or ketonemic are presented in Table 1.

Preinfection parameters of ketonemic and nonketonemic cows are summarized in Table 2. Differences between both groups in the average preinfection chemotactic response of PMNL in WBC suspensions, number of circulating leukocytes and PMNL, rectal temperature, heart rate, and milk production were not significant. Average BHBA and glucose concentrations between groups were significantly different ($P < .001$).

Severity of Experimentally Induced *E. coli* Mastitis

Bacterial counts in the inocula ranged from 40 to 57 cfu/ml, which corresponded to a total inoculum of 800 to 1140 cfu per quarter. At d 5 postinoculation, 1 cow (number 8) developed acute necrotic *Staphylococcus aureus* mastitis in the right front quarter and was euthanatized. All data collected from this cow were used until her death.

The AUC showed good correlations with clinical parameters and milk production during the experimental mastitis (Table 3). The correlation between AUC and log peak bacterial count was .86 ($P < .005$).

Results of the analysis of the relationship between severity of experimental mastitis and chemotactic response of PMNL in WBC suspensions just prior to inoculation (corrected for BHBA concentration in the peripheral blood) are presented in Table 4. We concluded that,

TABLE 1. Concentrations of BHBA and glucose in serum at inoculation, the severity of the experimental *Escherichia coli* mastitis in unrestricted and feed-restricted cows, and the classification in ketonemic and nonketonemic cows.

Cow	BHBA	Glucose	AUC[1]	Ketonemic
	(mmol/L)		(log_{10} cfu/h)	
Unrestricted				
1	2.61	2.5	525	Yes
2	.95	2.8	541	No
3	.91	2.6	297	No
4	.87	2.7	273	No
5	.76	2.8	690	No
6	1.09	2.7	638	No
7	.80	2.6	199	No
8	4.20	2.1	646	Yes
9	4.85	1.8	606	Yes
10	4.50	1.6	611	Yes
11	2.24	2.2	298	Yes
12	1.01	2.6	356	No
Feed restricted				
1	3.15	1.7	438	Yes
2	3.55	1.9	590	Yes
3	2.77	1.8	609	Yes
4	3.37	2.0	503	Yes
5	4.07	1.6	598	Yes
6	2.47	2.2	482	Yes

[1]Area under the curve.

TABLE 2. Mean chemotactic response of white blood cells, number of circulating leukocytes and neutrophils, BHBA and glucose concentrations in the peripheral blood at inoculation, baseline rectal temperature, baseline heart rate, and baseline total milk production in 11 ketonemic and 7 nonketonemic cows.

Preinfection parameter	Ketonemic		Nonketonemic	
	$\bar{X}$	SD	$\bar{X}$	SD
Chemotactic differential, mm	5.1	1.8	4.0	2.1
Chemotactic index	7.8	4.1	6.6	3.0
Number of leukocytes, $\times 10^9$/L	6.3	2.2	6.3	1.9
Number of neutrophils, $\times 10^9$/L	2.0	.9	2.1	1.1
BHBA, mmol/L	3.4	.9	.9	.1
Glucose, mmol/L	1.9	.3	2.7	.1
Rectal temperature, °C	38.7	.2	38.7	.2
Heart rate, beats per min	77.5	7.7	77.7	7.0
Total milk production	24.0	4.9	26.1	5.1

when BHBA concentrations in the peripheral blood are relatively high, no relationship existed between severity of experimental mastitis and chemotactic response of PMNL in WBC suspensions. The relationship between severity of experimental mastitis and chemotactic response of PMNL in WBC suspensions was significant when BHBA concentrations were relatively low. When the chemotactic responses of PMNL in WBC suspensions isolated 24 and 48 h before inoculation were used in the model, the same pattern can be described.

For all ketonemic cows (BHBA >1.4 mmol/L), the course of the experimental mastitis was relatively severe (Table 1; Figure 1). No relationship exists between the chemotactic differential of PMNL in WBC suspensions at 48 h, 24 h, or just before inoculation and the severity of the experimental mastitis (Table 5). Severity of experimental mastitis in the nonketonemic cows (BHBA <1.4 mmol/L) ranged from moderately to severely diseased (Table 1; Figure 2). In these nonketonemic cows, the chemotactic differential at 48 h, 24 h, and just before inoculation was significantly and negatively related to the severity of the disease (Table 5).

The correlation between the chemotactic index at 48 h, 24 h, and just before inoculation and the severity of the experimental mastitis was not significant for either group (Table 5). A significant negative correlation exists between the number of circulating PMNL just

TABLE 3. Correlation between area under the curve ($\log_{10}$ bacterial counts vs. time); milk production in control and infected quarters; rectal temperatures; and heart rates at d 1, 2, 3, 4, 5, and 14 postinoculation (n = 18).

Time post-inoculation	Quarter milk production[1]		Rectal temperature[2]	Heart rate[2]
	Control	Infected		
(d)				
1	−.72***	−.45	.67***	.68***
2	−.73***	−.56**	.67***	.50*
3	−.70***	−.80***	.32	. . .
4	−.64***	−.83***	.17	.24
5	−.50*	−.77***	.21	.40
14	.16	−.86***	. . .	. . .

[1]Percentage of baseline values.

[2]Difference from baseline values.

*$P < .05$.

**$P < .01$.

***$P < .005$.

TABLE 4. Results of the regression of the area under the curve.[1]

Parameter[2]	CV	SE	P
Intercept	637.6	77.0	.000
CD	-59.1	13.3	.0006
BHBA, mmol/L	14.31	31.5	.66
CD × BHBA	37.95	16.6	.03

[1]Adjusted R^2 of this model = .63.

[2]CD = Chemotactic differential.

prior to inoculation and the severity of experimental mastitis in the nonketonemic cows (Table 5). For the ketonemic cows, no correlation existed between the number of circulating PMNL prior to inoculation and the severity of experimental mastitis (Table 5).

DISCUSSION

In dairy cows, in vivo experiments on the association between clinical ketosis and increased susceptibility to mastitis are problematic (5). Mills et al. (22) reported that feed restriction would not sustain increased concentrations of BHBA in the peripheral blood or cause clinical ketosis. As shown in the present study and in other studies (2, 10, 20), feed restriction induced increased BHBA concentrations at the onset of experimental mastitis in most cows. In all feed-restricted cows, the BHBA concentration was >1.4 mmol/L after 4 d of feed restriction. A concentration of >1.4 mmol/L was considered to be indicative for severe energy deficit in early lactating cows (4). All cows had plasma glucose concentrations exceeding those in cows with clinical ketosis (Table 1). Glucose concentrations of .8 to 1.4 mmol/L are typical of those during naturally occurring clinical ketosis. Therefore, the feed-restriction model is not appropriate for investigation of metabolic events occurring during development of clinical ketosis or the interrelated fatty liver syndrome (5, 6, 22, 29). However, with these limitations, the feed-restriction model can be used for studies investigating the influence of the role of increased BHBA concentrations or other events associated with negative energy balance on PMNL function and severity of infections (1, 9, 20).

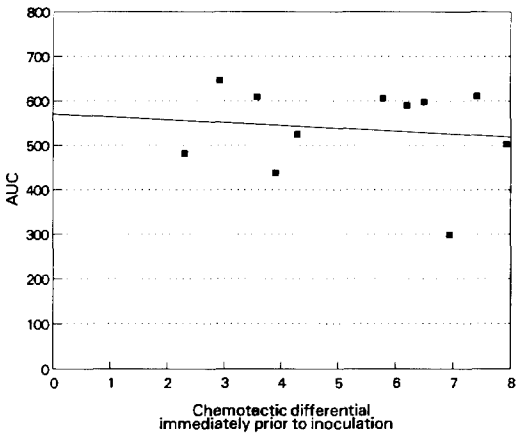

Figure 1. Relationship between chemotactic differential of white blood cells, isolated immediately prior to inoculation, and area under the curve (AUC) (bacterial count vs. time) in ketonemic cows. Y = 571.2 − 6.5(chemotactic differential + error).

Five of 12 nonrestricted cows had BHBA concentrations >1.4 mmol/L; thus, the cows used in our study were very susceptible to energy deficit, resulting in increased BHBA concentrations in the peripheral blood. This susceptibility may not be related only to the high milk production of these early lactating cows; events during and after transportation to the research institute might also have contributed to the increased susceptibility to ketonemia.

For the nonketonemic cows, severity of experimental mastitis ranged from moderate to severe. The experimental mastitis was related to the preinfection chemotactic response of PMNL in WBC suspensions (Table 5; Figure 2), as previously reported (18). In ketonemic cows, the course of experimental *E. coli* mastitis was relatively severe, and the severity of the experimental mastitis was unrelated to

TABLE 5. Correlation between chemotactic response of white blood cells immediately prior to inoculation, at d 1 and 2 before inoculation, the number of circulating polymorphonuclear leukocytes (PMNL) prior to inoculation, and the area under curve (log_{10} bacterial counts vs. time) in ketonemic (n = 11) and nonketonemic cows (n = 7).

Preinfection parameter	Before inoculation	r	
		Ketonemic	Nonketonemic
	(d)		
Chemotactic differential	0	−.11	−.85**
	1	.05	−.90***
	2	−.16	−.92***
Chemotactic index	0	−.16	−.59
	1	−.31	−.66
	2	−.53	−.71
PMNL, no.	0	−.23	−.87**

**$P < .01$.

***$P < .005$.

PMNL chemotactic response (Table 5; Figure 2). Glucose and BHBA and concentrations in the peripheral blood were highly correlated at inoculation ($r = .9$). Therefore, the same pattern could be described when glucose was used in the regression model (data not presented). Changes that were associated with negative energy balance, increased BHBA concentrations, or decreased glucose concentrations in the peripheral blood possibly overrule the effect of PMNL chemotactic response. These changes may affect directly or indirectly the functional capacity of the immune system. Poorly understood are the physiological mechanisms by which the immune system is influenced by negative energy balance or the role of increased BHBA or decreased glucose concentrations. Some in vitro studies have demonstrated an inhibitory effect of ketone concentrations associated with clinical ketosis on in vitro leukocyte function (10, 16, 17, 27). In a recent in vivo study, Filar et al. (9) suggested that interferon production was impaired in feed-restricted cows with increased BHBA concentrations.

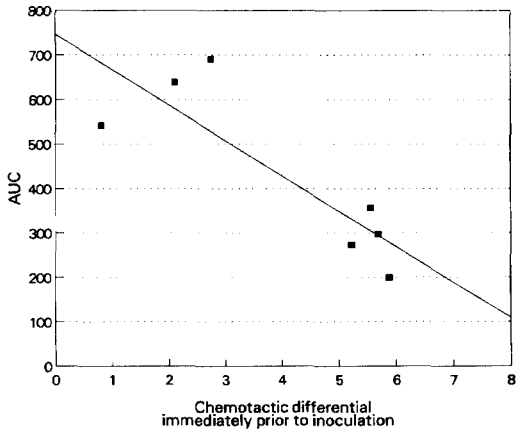

Figure 2. Relationship between chemotactic differential of white blood cells, isolated immediately prior to inoculation, and area under the curve (AUC) (bacterial count vs. time) in nonketonemic cows. Y = 746.1 − 79.6(chemotactic differential + error).

Leukopenia has been associated with ketosis and fatty liver syndrome (26). However, in the present study, no relationship exists between number and distribution of leukocytes and the BHBA concentration in ketonemic cows. In nonketonemic cows, the number of circulating PMNL immediately prior to inoculation was negatively related to the AUC (Table 5), as has been reported (12, 18).

CONCLUSIONS

The course of experimental *E. coli* mastitis in ketonemic cows was relatively severe, regardless of the preinfection PMNL chemotactic response. Changes associated with negative energy balance appear to be more important in determining the outcome of an experimental mastitis than PMNL chemotactic response. In contrast, severity of experimental mastitis ranged from moderate to severe in the nonketonemic cows. The chemotactic response of PMNL in WBC suspensions was negatively

related to the severity of experimental mastitis in nonketonemic cows (18). Additional research is necessary to explain the exact mechanisms that compromise the bovine immune system in negative energy balance during early lactation resulting in increased susceptibility to infectious diseases.

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REFERENCES

1 Baird, G. D. 1982. Primary ketosis in the high producing dairy cow: clinical and subclinical disorders, treatment, prevention, and outlook. J. Dairy Sci. 65:1.

2 Baird, G. D., R. J. Heitzman, and K. G. Hibbit. 1972. Effects of starvation on intermediary metabolism in the lactating cow. A comparison with metabolic changes occurring during bovine ketosis. Biochem. J. 128:1311.

3 Burvenich, C., R. Heyneman, G. van de Putte-van Messom, A. M. Massart-Leën, and E. Roets. 1989. Differences in local and systemic reactions during severe and moderate cases of *Escherichia coli* mastitis in cattle soon after parturition. Proc. Int. Conf. Mastitis, Austria, May 29–June 2, 1989.

4 Compendium Laboratory Diagnostics of National Animal Health Committee. 1992. Natl. Anim. Health Committee, Prinsevinkenpark 24, 2585 HL. The Hague, Neth.

5 Drackely, J. K., M. J. Richard, D. C. Beitz, and J. W. Young. 1992. Metabolic changes in dairy cows with ketonemia in response to feed restriction and dietary 1,3-butanediol. J. Dairy Sci. 75:1622.

6 Drackley, J. K., J. J. Veenhuizen, M. J. Richard, and J. W. Young. 1991. Metabolic changes in blood and liver of dairy cows during either feed restriction or dietary 1,3-butanediol. J. Dairy Sci. 74:4254.

7 Eberhart, R. J., R. P. Natzke, F.H.S. Newbould, B. J. Nonnecke, P. Thompson. 1979. Coliform mastitis—a review. J. Dairy Sci. 62:1.

8 Erb, H. N., and Y. T., Gröhn. 1988. Epidemiology of metabolic disorders in the periparturient dairy cow. J. Dairy Sci. 74:2507.

9 Filar, J., M. Kandefer-Szerszen, A. Szuster-Ciesielska, and W. Rzeski. 1992. Effect of cold treatment and ketosis induced by starvation on interferon production in leukocytes of lactating cows. Dtsch. Tieraerztl. Wochenschr. 99:210.

10 Franklin, S. T., J. W. Young, and B. J. Nonnecke. 1991. Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. J. Dairy Sci. 74:2507.

11 Gröhn, Y. T., H. N. Erb, C. E. McCulloch, and H. Sloneimi. 1989. Epidemiology of metabolic disorders in dairy cattle: association among host characteristics, disease, and production. J. Dairy Sci. 72:1876.

12 Heyneman, R., C. Burvenich, and R. Vercauteren. 1990. Interaction between the respiratory burst activity of neutrophil leukocytes and experimentally induced *Escherichia coli* mastitis in cows. J. Dairy Sci. 73:985.

13 Hill, A. W. 1981. Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow. Res. Vet. Sci. 31:107.

14 Hill, A. W., A. L. Shears, and K. G. Hibbit. 1979. The pathogenesis of experimental *Escherichia coli* mastitis in newly calved cows. Res. Vet. Sci. 26:97.

15 Jain, N. C., O. W. Schalm, and E. J. Carroll. 1968. Experimental mastitis in leukopenic cows: immunologically induced neutropenia and response to intramammary inoculation of *Aerobacter aerogenes*. Am. J. Vet. Res. 29:2089.

16 Klucinski, W., W. Degorski, E. Miernik-Degorska, S. Targowski, and A. Winnicka. 1988. Effect of ketone bodies on phagocytic activity of bovine milk macrophages and polymorphonuclear leukocytes. J. Vet. Med. A35:632.

17 Klucinski, W., E. Miernik-Degorska, W. Degorski, S. Targowski, and A. Winnicka. 1988. Effect of ketone bodies on mitogenic response of bovine milk lymphocytes. J. Vet. Med. A35:626.

18 Kremer, W.D.J., E. N. Noordhuizen-Stassen, F. J. Grommers, A.J.J.M. Daemen, P.A.J. Henricks, C. Burvenich, and A. Brand. 1993. Preinfection chemotactic response of blood polymorphonuclear leukocytes as a predictor of the severity of experimental *Escherichia coli* bovine mastitis. J. Dairy Sci. 76: 2514.

19 Kremer, W.D.J., E. N. Noordhuizen-Stassen, P.A.J. Henricks, and H. van der Vliet. 1992. A procedure for parallel isolation of white blood cells and purified neutrophil suspensions from the peripheral blood of cattle. Vet. Immunol. Immunopathol. 31:189.

20 Kronfeld, D. S. 1971. Hypoglycemia in ketotic cows. J. Dairy Sci. 54:949.

21 Lohuis, J.A.C.M., Y. H. Schukken, P.A.J. Henricks, R. Heyneman, C. Burvenich, J.H.M. Verheyden, A.S.J.P.A.M. vanMiert, and A. Brand. 1990. Preinfection functions of blood polymorphonuclear leukocytes and the outcome of experimental *Escherichia coli* mastitis in the cow. J. Dairy Sci. 73:342.

22 Mills, M. J., D. C. Beitz, and J. W. Young. 1986. Characterization of metabolic changes during a protocol for inducing lactation ketosis in dairy cows. J. Dairy Sci. 69:352.

23 Nelson, R. D., P. G. Quie, and R. L. Simmons. 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis of and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J. Immunol. 115:1650.

24 Nonnecke, B. J., S. T. Franklin, and J. W. Young. 1992. Effects of ketones, acetate, and glucose on in vitro immunoglobulin secretion by bovine lymphocytes. J. Dairy Sci. 75:982.

25 Paape, M. J., A. J. Guidry, N. C. Jain, and R. H. Miller. 1991. Leukocytic defense mechanisms in the udder. Flem. Vet. J. 62(Suppl. 1):95.

26 Reid, I. M., A. M. Dew, and L. A. Williams. 1984. Haematology of subclinical fatty liver in dairy cows. Res. Vet. Sci. 37:63.

27 Sato, N., H. Shimizu, Y. Shimomura, K. Suwa, M. Mori, and I. Kobayashi. 1992. Mechanism of inhibi-

tory action of ketone bodies on the production of reactive oxygen intermediates (ROIS) by polymorphonuclear leukocytes. Life Sci. 51:113.

28 Targowski, S. P., W. Klucinski, E. T. Littledike, and D. A. Hoy. 1985. Suppression of mitogenic response of bovine lymphocytes during experimental ketosis in calves. Am. J. Vet. Res. 46:1378.

29 Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74:4238.