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Witaya Suriyasathaporn, Cord Heuer, Elsbeth Noordhuizen-Stassen, Ynte Schukken. Hyperketonemia and the impairment of udder defense: a review. *Veterinary Research*, 2000, 31 (4), pp.397-412. 10.1051/vetres:2000128 . hal-00902662

HAL Id: hal-00902662

<https://hal.science/hal-00902662>

Submitted on 11 May 2020

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Review article

Hyperketonemia and the impairment of udder defense: a review

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(Received 10 December 1999; accepted 6 March 2000)

Abstract – The objective of this study was to review the possible relationships between hyperketonemia and the function of phagocytes with respect to the bovine udder defense mechanism. We hypothesize that an increased incidence of clinical mastitis in high-producing cows is caused by the impairment of the udder defense mechanism during hyperketonemia. First, we review the acute phase of udder defense mechanisms after intramammary infection. The physiological changes of cows in negative energy balance are subsequently discussed. Finally, possible relationships between udder defense and physiological changes during negative energy balance, especially hyperketonemia, are reviewed. The three stages of an acute phase of udder defense are: (1) immediately eliminating invading pathogens by phagocytes, (2) releasing inflammatory substances, especially chemoattractants, and (3) migration of polymorphonuclear leukocytes into the infected udder. Leukocytes from hyperketonemia subjects show a lower capacity of the phagocytic defense mechanism. In addition, the phagocytic and bactericidal capacities of neutrophils are reduced when these cells are acting in the presence of high concentrations of ketone bodies. Lower amounts of cytokine production after bacterial infection are observed in ketotic subjects. The chemotactic capacity of blood leukocytes is impaired in leukocytes obtained from ketotic cows. Lower numbers of blood leukocytes are observed in ketotic cows. In conclusion, the impairment of the udder defense mechanism in negative energy balance cows seems related to hyperketonemia.

ketosis / negative energy balance / mastitis / udder defense mechanism / cow

Résumé – **Hypercétonémie et diminution des défenses mammaires : une synthèse.** L'objectif de cette étude est de présenter des relations possibles entre l'hypercétonémie et la fonction des phagocytes pour ce qui est des mécanismes de défense de la mamelle. Notre hypothèse est qu'une

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augmentation des mammites cliniques chez des vaches laitières à haute production serait causée par une détérioration des mécanismes de défense de la mamelle suite à une hypercétonémie. Premièrement, le stade aigu des mécanismes de défense de la mamelle après une infection a été étudié. Ensuite, les changements physiologiques chez les vaches ayant un bilan énergétique négatif ont été présentés. Finalement, les relations entre les mécanismes de défense de la mamelle et les changements physiologiques au cours d'un bilan énergétique négatif, notamment une hypercétonémie, ont été revus. Les trois stades d'une phase aiguë de défense mammaire sont les suivants : (1) des organismes pathogènes envahisseurs sont phagocytés immédiatement, (2) des substances inflammatoires sont produites, en particulier des substances chimio-tactiques, (3) des leucocytes polynucléaires migrent vers la mamelle infectée. La capacité phagocytaire des leucocytes ainsi que celle des neutrophiles et la capacité bactéricide de ceux-ci sont apparemment détériorés dans les conditions d'hypercétonémie. La quantité de cytokines produites après une infection bactérienne est diminuée chez les sujets hypercétonémiques. Le chimiotactisme des leucocytes sanguins est détérioré dans les leucocytes provenant de vaches hypercétonémiques. Des quantités plus faibles de leucocytes sanguins sont observés dans les vaches hypercétonémiques. En conclusion, la détérioration des mécanismes de défense de la mamelle au cours de la période du bilan énergétique négatif semble liée à l'hypercétonémie.

hypercétonémie / bilan énergétique négatif / mammite / mécanismes de défense de la mamelle / vache

Table of contents

1. Introduction	398
2. An acute phase of udder defense mechanism after intramammary infection	399
3. Ketogenesis in negative energy balance cows during the postpartum period	401
4. Hyperketonemia and udder defense mechanism	403
4.1. Capacity of the phagocytosis by polymorphonuclear cells and macrophages.....	403
4.2. Capacity of udder leukocytes to induce cell recruitment.....	404
4.3. Capacity of blood leukocytes to migrate into the infected gland	404
5. Mechanistic relationships between hyperketonemia and udder defense	405
6. Conclusion.....	406

1. INTRODUCTION

As with most infectious diseases, mastitis incidence depends on three components: exposure to microbes, udder defense mechanisms, and environmental risk factors. Udder defense plays a role in protecting and minimizing the expression of the clinical symptoms in infected mammary glands [52]. Regardless of bacterial species, both quantity and quality of polymorphonuclear leukocytes (PMN) in the udder are important components of udder defense in order to protect cows from clinical mastitis [10, 11, 18, 46, 72, 97, 109]. In experimental mastitis challenge studies, cows with lower chemotactic capacities of blood PMN also had a higher severity of mastitis indicated by bacteria counts in milk [13, 56, 57, 115].

Over the past 35 years, milk production has dramatically increased in Western Europe and North America. An increased milk yield means an increased profitability for farmers. However, several studies have demonstrated that high-producing cows are at an increased risk of infectious diseases [21, 33, 34, 74, 75, 112]. Among these diseases, clinical mastitis is the most costly disease of the dairy industry [26] due to decreased production, costs of treatment, extra labor, and an increased rate of cow replacement [5]. An antagonistic genetic correlation between milk production and risk of disease showed that the improvement of milk production consequently increased the incidence of clinical mastitis [104, 110, 112].

Negative energy balance (NEB) has been considered an important problem in high-producing dairy cows. Several factors including body condition score (BCS), serum non-esterified fatty acids (NEFA), the fat/protein ratio in milk, and ketone bodies are signs of NEB [43, 59, 91]. In epidemiological studies, ketosis is associated with an increased risk of clinical mastitis [15, 24, 74]. However, it is not clear to date by what mechanism hyperketonemia interferes with udder defense. Therefore, the aims of the present study were to review the mechanisms that have been suggested in the literature. Firstly, we describe the role of PMN in the acute phase of udder defense mechanisms after intramammary infection (IMI). This review will not address the differences among pathogens, even though some immune responses are different among pathogens, especially the capacity of pathogen recognition by phagocytes [39, 62]. In relation to NEB, ketogenesis in early lactation is reviewed in the second part. Finally, based on the relevant literature, we suggest possible mechanistic relationships between hyperketonemia and udder defense.

2. AN ACUTE PHASE OF UDDER DEFENSE MECHANISM AFTER INTRAMAMMARY INFECTION

Several researchers have reviewed the normal physiological processes of udder defense mechanisms [10, 16, 55, 106]. After bacterial invasion, leukocytes in milk recognize pathogens, and innate immunity attempts to resolve this IMI immediately (Fig. 1A). In case innate immunity cannot resolve IMI, an early inflammatory response by the mammary gland is induced between 4–96 h after infection (Figs. 1B and 1C). Leukocytes and epithelial cells in the infected quarters release products, many of which are chemoattractants for leukocytes (Fig. 1B). Neutrophils move rapidly from the blood stream into the infected quarters (Fig. 1C). If the bacteria are destroyed, recruitment of neutrophils into the gland is ceased, and only a mild inflammatory episode will be required to restore health in the gland [52]. Occasionally, the defense mechanisms of the infected mammary gland lose the battle with bacteria, and the bacteria multiply. Various cell types in the udder produce abundant soluble factors, such as

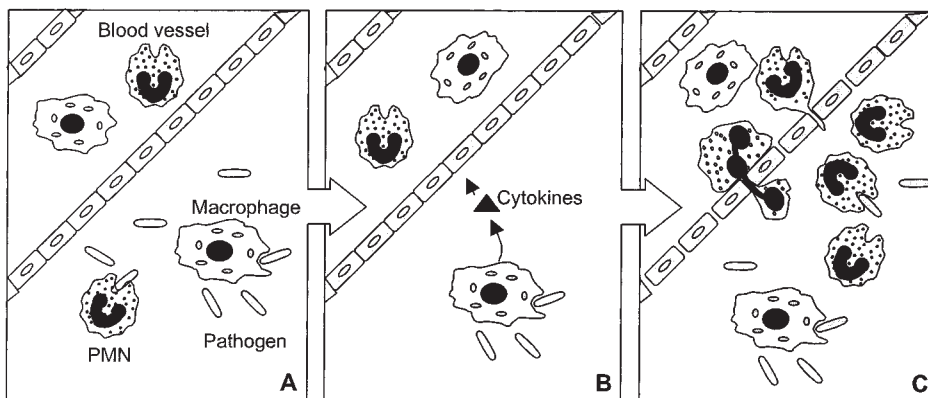


Figure 1. Three components of an acute phase of udder defense mechanisms after intramammary infection: (A) elimination of invading pathogens immediately by phagocytes, (B) release of inflammatory substances, especially chemoattractants, and (C) migration of polymorphonuclear leukocytes into infected udder.

cytokines, that cause clinical signs of mastitis.

Innate immunity in the udder is composed of humoral and cellular factors. Humoral factors include complement, lactoferrin, lysozyme, and the lactoperoxidase-thiocyanate-hydroperoxide system [55]. Cellular factors usually refer to udder phagocytes. Udder phagocytes, usually PMN, act to remove the invading pathogen by specific processes that include opsonization, ingestion, and killing. Efficiency of pathogen removal is related to either the quantity or quality of phagocytes in the udder [18]. Because phagocytes, which are composed of 12–26% of PMN and 30–74% of macrophages, are the main component of the somatic cell count (SCC) in the uninfected gland, a very low SCC is related to an increased risk of clinical mastitis [97, 109]. In experimental mastitis challenge studies, the severity of mastitis, as indicated by bacterial counts, is increased in cows with low pre-infection SCC [18, 96, 102, 114].

Because 90% of PMN in infected glands are the PMN recruitment from blood vessel after IMI [65], blood PMN has been used to evaluate the role of udder immunity. The severity of clinical mastitis after IMI is, however, less dependent on the functional capacity of blood phagocytes [3, 11, 115] than on the intra-mammary functional capacity of these cells. In the mammary gland, a low efficiency of PMN phagocytosis is caused by either ingestion of milk constituents, such as fat globules [25, 76] and casein micelles [92, 93], or a deficiency of opsonins in milk [76, 83, 85]. Consequently, an increase of opsonins, such as immunoglobulins, results in a lower incidence of clinical mastitis [36, 47, 50]. After ingestion by PMN, microorganisms are subject to destructive mechanisms. Two mechanisms have been described: an oxygen-dependent mechanism and an oxygen-independent one. Although myeloperoxidase with oxidizing agents form a potent antibacterial system against common udder pathogens, it loses its bactericidal activity

in milk containing bacterial cultures [14]. A deficiency in the destructive mechanism capacity, indicated by luminol-enhanced chemiluminescence, was demonstrated in PMN of cows developing mastitis compared to healthy controls [14, 118]. The generation of reactive oxygen species is also related to the severity of experimental IMI [44, 61].

After phagocytes are activated against invading bacteria, bacteria and leukocytes present in the infected quarters release or take part in generation of products, such as endotoxin or lipopolysaccharide (LPS) [78, 79], complement (C5a) [78, 82, 87, 103, 105], C3 [66, 84, 86], interleukin-1 (IL-1) [79–81, 100–103], IL-6 [68, 100], IL-8 [4, 7, 80, 102, 103], and tumor necrosis factor (TNF- α) [103]. However, milk macrophages from healthy and mastitic quarters secrete a small quantity of IL-1 compared to blood monocytes [81]. These products are mediators of inflammation, comprising pro-inflammatory cytokines, such as IL-1 [17, 73] and TNF- α [78], and chemoattractants, such as IL-8 [4, 7, 103] and C5a [78, 103, 105]. These substances have been used for prevention and treatment of chronic mastitis [17, 19, 20, 88, 94]. Several studies have reviewed the application of cytokines to mastitis control [53, 71, 106].

In response to chemo-attractants, PMN adhere to and migrate along the endothelial cell surface towards the site of infection. Migration of blood PMN between endothelial cells into the site of infection is dependent on the number of blood PMN and its migration capacity [98, 114]. Low numbers of blood PMN pre-challenge are also related to increased severity of experimental *Escherichia coli* mastitis [57, 114, 115].

The capacity of blood leukocytes to migrate into the infected udder is essential in protecting the mammary gland from clinical *E. coli* mastitis [11, 46, 102, 115]. This parameter is associated with the capacity of in vitro chemotaxis of PMN [35]. Assays of in vitro chemotaxis of PMN have been established as determinants of the prevention

of clinical mastitis [11]. Also, *in vitro* chemotaxis was related to the severity of infection indicated by recurring bacterial counts throughout the course of disease [56, 57, 115].

The mechanism of migration of leukocytes out of blood vessels, or extravasation, depends on adhesive interactions activated by the chemo-attractant (Fig. 2). Without an exposure to the chemo-attractant, the interaction of the adhesive molecules between leukocytes and endothelial surface mediated by E- and P-selectin is weak, but the strong interaction appears after exposure to the chemo-attractant, as shown in Figure 2A. Then integrins on leukocytes, like LFA-1 (CD11a:CD18) and Mac-1 (CD11b:CD18), act on ICAM-1. In consequence, the leukocyte attaches firmly to the endothelium and the rolling is arrested (Fig. 2B). In the last step, leukocytes extravasate, and then migrate through the tissue under the influence of the chemo-attractant (Fig. 2C). This step is related to the expression of CD31 in leukocytes and endothelial cells. However, preinfection expression of CD11a and CD11b is not

related to the severity of mastitis [22, 115]. The absence of this relation may be due to the increased expression of integrin after infection [77, 105]. The lack of appearance of chemotactic and CD18-upregulating activities until 12 h after challenge indicate that delays in neutrophil recruitment result from an initial lack of bacterial recognition and inflammatory mediator production [103]. Therefore, the upregulation of integrin expression after infection is more closely related to the capacity of udder defense [102]. Some substances, for example cortisol, induce a decrease in the expression of CD18 receptors after experimental IMI, probably modulating the acute inflammatory response in mammary glands of lactating cows [90].

3. KETOGENESIS IN NEGATIVE ENERGY BALANCE COWS DURING THE POSTPARTUM PERIOD

Due to a rapid increase of milk production immediately after calving, cows require more energy for maintenance, milk pro-

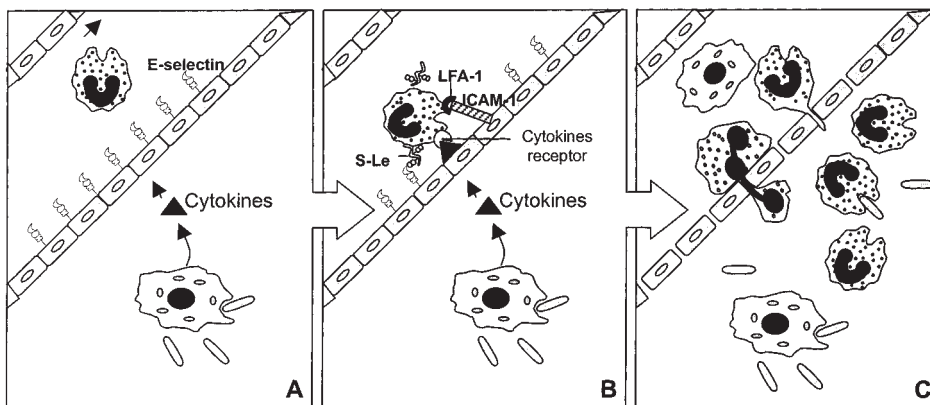


Figure 2. Migratory mechanism of blood PMN into infected udder: (A) expression of selectin after stimulation by chemoattractants such as cytokines, (B) rolling and adherence of blood leukocytes into endothelial wall mediated by selectin and integrin, and (C) invading and migration into the area of infection. LFA-1 (CD11a:CD18): leukocyte integrin, ICAM-1: the immunoglobulin related adhesion molecule on endothelium, and S-Le (the sialyl-Lewis moiety): the carbohydrate ligands on the leukocytes.

duction and growth than they are able to obtain through feed. Cows are in NEB until approximately 7–9 weeks of lactation whereas the milk production peak is already reached at 4–6 weeks (Fig. 3). Consequently, body reserves are utilized leading to a potential increase of ketone bodies in milk. In transition from the dry period to early lactation, mostly high-yielding cows come into a temporary state of physiological NEB. If feed quality, feed quantity, or the management of feed supply is suboptimal, this mild state of NEB may lead to severe, decompensated NEB, associated with acetonemia, and eventually to clinical ketosis. For example, cows calving in fat conditions may have lower feed intake [31]. An excess of rumen degradable protein in the ration requires extra energy for the removal of surplus ammonia from the rumen [1], and this can lead to a higher incidence of clinical ketosis [45]. The most frequent reason for decompensated NEB, however, is probably the insufficient preparation of ruminal microorganisms for the digestion of large amounts of starch after calving when the roughage

based ration of the dry period is replaced by substantial amounts of concentrate [32].

In response to NEB, hypoglycaemic cows decrease insulin and increase glucagon concentrations in blood [2]. The metabolism pathway of NEB cows is shown on Figure 4. Body fat reserves are mobilized and used as an additional source of energy for maintenance and milk production. Residual glycogen generally lasts only a few hours, hence fat reserves quickly become the sole source of energy soon after calving. Mobilization of fatty acid results in an increase of non-esterified fatty acids (NEFA) and glycerol in blood [2, 28, 89, 91, 113]. NEFA is taken up by the liver and enters the beta-oxidation pathway that results in the formation of NADH and acetyl-CoA in mitochondria [6, 58]. Acetyl-CoA is further oxidized by condensation with oxaloacetate that, however, is also the main substrate for gluconeogenesis. In a hypoglycemic state, gluconeogenesis has priority over the removal of acetyl-CoA. This results in the insufficient availability of a substrate for the

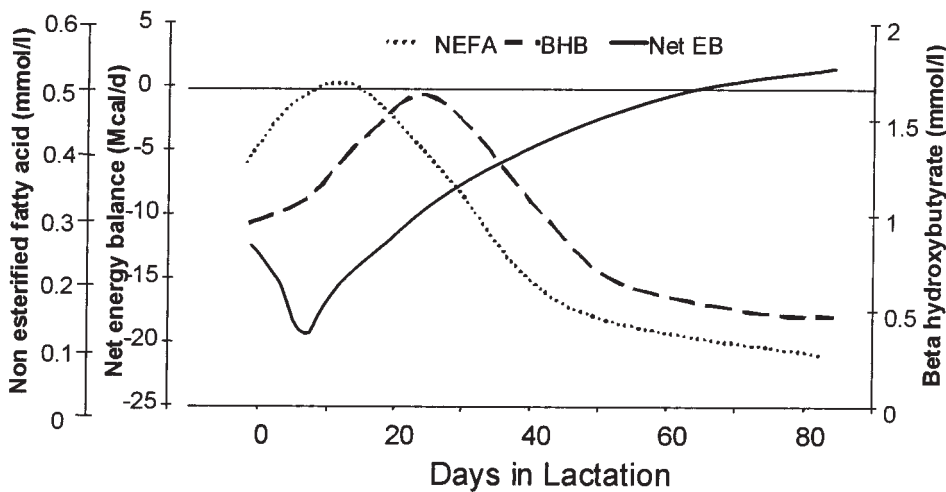


Figure 3. Level of energy balance in postpartum cows in relation to concentrations of nonesterified-fatty acid (NEFA) and β -hydroxybutyrate (BHB) [adapted from Lean et al. [59] and Rukkwamsuk et al. [91].

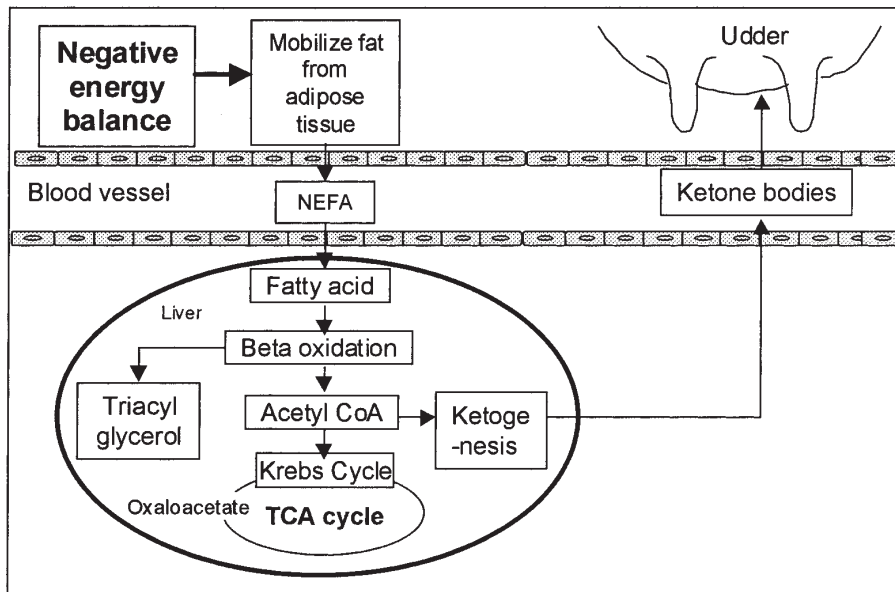


Figure 4. Metabolic pathways of ketogenesis in blood circulation and the udder. NEFA: Non-esterified fatty acid, TCA: Krebs's tricarboxylic cycle.

synthesis of acetyl-CoA to citrate. Hence, acetyl-CoA accumulates as long as the cows remain energy deficient.

Consequently, accumulated acetyl-CoA is removed via synthesis to the ketone body acetoacetyl-CoA which is further condensed to acetoacetate (AcAc) [6, 63]. After transport to several organs, AcAc is reduced in the mitochondria by transformation to β -OH-butyrate (BHB), and some is spontaneously and irreversibly decarboxylated to acetone. High concentrations of ketone bodies reduce feed intake, decrease the mobilization of fatty acids and consequently, aggravate energy deficiency [9, 41, 42, 113]. As the result of decompensated NEB, the concentrations of BHB, AcAc, and acetone in blood and milk increase. We suggest that these ketone bodies negatively interact with udder defense mechanisms.

4. HYPERKETONEMIA AND UDDER DEFENSE MECHANISM

According to our description of udder defense, the success of the prevention of clinical mastitis depends on (1) the phagocytosis by phagocytes in udder, (2) capacity to induce cells recruitment, and (3) the capacity of blood leukocytes to migrate into the infected gland (Fig. 1). Therefore, we describe the effects of NEB on these udder defense mechanisms separately.

4.1. Capacity of the phagocytosis by polymorphonuclear cells and macrophages

Several authors have reviewed the factors affecting the SCC in dairy cows [40, 52, 60], but they are intensively related to SCC after IMI. van Werven [114] has shown

that SCC from an uninfected udder is less related to the number of blood leukocytes. Suriyasathaporn et al. [109] have shown a positive correlation between SCC in mastitis-free cows and body condition score. This would indicate a somewhat low SCC in NEB cows.

When cows have severe NEB, udder leukocytes have to ingest and kill invading pathogens in the presence of high ketone bodies. Klucinski et al. [54] found that the phagocytosis of bacteria by milk PMN and macrophages that are incubated in acetone or BHB are lower than in cell cultures without ketone bodies. It has also been reported in a study on patients with insulin-dependent diabetes, that BHB in cultures inhibit neutrophil phagocytosis and killing of *Candida albicans* in vitro [117]. In heparinized blood PMN of humans, the average number of ingested bacteria is reduced by prolonged exercise [30], indicating that acute NEB reduce the PMN phagocytosis capacity. However, the phagocytosis of neutrophils after prolonged NEB as observed in obese patients with dietary restrictions is unchanged, and this is even the case in neutrophils obtained after 3 months of feed restriction during which symptoms of urinary ketosis occur [64].

A study of leukocytes from obese persons has shown that the killing capacity is reduced when the leukocytes are cultured in sera from subjects with ketonuria [64]. After prolonged exercise, the amount of superoxide anions produced by leukocytes is lower in NEB humans [29, 38]. Concentration of ketone bodies is negatively associated with the generation of superoxide anions and with respiratory burst activity [29]. Furthermore, PMN from blood of type I diabetes mellitus patients show a reduced respiratory burst activity after pre-incubation with higher amounts of BHB [99]. The leukocytes from cows also show a suppression of respiratory burst activity when cultured in the presence of BHB [48]. These workers suggested that BHB causes this suppression by inhibiting the generation of

superoxide anions, because respiratory burst activity regulates the production of superoxide anions.

While it may be controversial whether or not ketone bodies reduce the capacity of leukocytes for phagocytosis in vivo, several studies have clearly indicated that the killing capacity of leukocytes is impaired by ketone bodies in vitro and in vivo.

4.2. Capacity of udder leukocytes to induce cell recruitment

The function of blood and milk lymphocytes in response to mitogens is impaired in ketotic cows [51, 95, 111]. NEB is closely related to hepatic lipidosis [91], and induced-hepatic-lipidosis cows have lower IgG levels two weeks after tetanus toxoid immunization than control cows [116]. Hence, NEB appears to interfere with the production of opsonins (like IgG) which are necessary for phagocytosis. After IMI, leukocytes and bacteria directly or indirectly induce the migration of blood leukocytes to the site of infection and thus mediate the development of inflammation. Ketotic cows have lower amounts of cytokines produced by lymphocytes, such as interferons, than normal cows [27, 51]. After stimulation with LPS, the production of several inflammatory mediators, such as IL-1 β and TNF- α , decreases when cultured with whole blood from humans in NEB [23]. Blood lymphocytes from NEB cows with ketonemia have lower mitogenic responses than lymphocytes from non-ketonemic cows [27, 51, 95, 111]. Therefore, we may hypothesize that the generation of chemoattractant is reduced in hyperketonemic cows.

4.3. Capacity of blood leukocytes to migrate into the infected gland

Among factors related to NEB (BHB, glucose, insulin, and NEFA), only the concentration of BHB shows a strong positive

correlation to the severity of experimental mastitis, indicated by bacteria count [114]. The authors have demonstrated that the BHB level is related to the preinfection number of blood leukocytes and the magnitude of leukocyte influx in the experimentally infected mammary glands. In an *in vitro* study, BHBA and AcAc added in culture media induce inhibitory effects on the proliferation of bovine bone marrow cells [49].

The magnitude of leukocyte influx is dependent on the chemotactic capacity of leukocytes. The relationship between NEB, ketone bodies and chemotaxis of blood PMN has been investigated [8, 37, 56, 64, 107, 108]. Sera from NEB humans with urinary ketosis inhibit leukocyte chemotactic response *in vitro* [64]. *In vitro* chemotactic differential and chemotactic index of blood PMN are higher in induced ketotic cows than in non-ketotic cows, though the difference is not significant [56]. These results are in agreement with studies of NEB in humans in whom the chemotactic capacity and the random migration of PMN from short-period NEB are not different in comparison with non-NEB subjects [8, 38]. However, cows with spontaneous postpartum NEB, in which it is assumed that the NEB occurs over several weeks, have impaired chemotactic functions of leukocytes [107, 108]. This is in agreement with a study on chemotaxis in NEB humans with 90 days of strenuous exercise and caloric deficiency. Compared to controls, the neutrophils of NEB subjects have a lower migration distance [8]. These studies indicate that especially long-term NEB or clinical ketosis is negatively associated with neutrophil chemotaxis.

Impairment of leukocyte chemotaxis has also been demonstrated in leukocytes acting in a ketone body environment [108]. Leukocytes in cultures supplemented with BHB, AcAc, acetone, and their mixture show less chemotactic capacity than leukocytes in the control culture. A decrease in the chemotactic index in cultures supplemented with ketone bodies indicates a lower

response to chemoattractants. Independently of chemotaxis, random migration of leukocytes from non-ketotic cows in cultures containing ketone bodies is significantly decreased, and this effect is also present, though weaker, in leukocytes from ketotic cows [107].

5. MECHANISTIC RELATIONSHIPS BETWEEN HYPERKETONEMIA AND UDDER DEFENSE

From this review, it becomes clear that leukocytes from NEB cows showed impaired activity in most steps of the udder defense mechanisms against mastitis (Tab. I). Consequently, NEB cows are related to an increase of the risk of clinical mastitis. The number of leukocytes in ketotic cows were lower than that of healthy cows. Leukocytes from cows with postpartum NEB had a slower response in producing chemoattractants, a lower migration rate, and a lower production capacity for superoxide anions. When the NEB period is short, however, the chemotaxis of the leukocytes is not affected [8, 56]. Therefore, the functional capacity of leukocytes may depend on the duration and severity of postpartum NEB. In a study in humans, the concentrations of glucose-6-phosphate dehydrogenase, pyruvate kinase, and adenosine kinase in leukocytes show minimal values during the puerperium [12]. Because the random movement and the production of superoxide anions by leukocytes requires large amounts of energy, the adverse effects of energy metabolism may be related to the impairment of leukocyte function in NEB cows. In addition, AcAc or BHB in culture may also interfere with the utilization of glucose for energy. In mouse models, AcAc or BHBA that are added in culture media are not utilized by mouse macrophages [69, 70].

Concentrations of BHB, AcAc, acetone, and free fatty acids are elevated in sera and milk of NEB cows. Consequently, leukocytes must function in high concentrations of

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

Udder defense mechanisms	Functional capacity	
	Leukocytes from ketotic subjects	Leukocytes cultured in the media supplemented with ketone
Phagocytic defense		
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		
Oponin	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

BHB: β -Hydroxybutyrate.

NEB: Negative Energy Balance.

these substances. Because ketone bodies are organic molecules, they might interfere with the attachment of inflammatory substances at the surface of leukocytes. In our *in vitro* underagarose chemotaxis study (unpublished results), concentrations of leukocytes in plates using either leukocytes from ketotic cows (Fig. 5C) or leukocytes in cultures supplemented with ketone bodies (Fig. 5B) were less than those in plates of leukocytes from healthy cows (Fig. 5A). The leukocytes from ketotic cows (Fig. 5C) or cultured in ketone bodies (Fig. 5B) were round and less firmly attached to the plastic dish. As capacity of *in vitro* chemotaxis is related to the expression of CD18 of PMN expressed by the firm attachment to the surface of a plastic well [67], the impairment of attachment to plastic well in our finding may relate to the impairment of expression of the adhesion molecules of leukocytes.

6. CONCLUSION

Cows in the NEB period show an impairment of udder defense mechanisms. Hyperketonemia seems to be one of the most important parameters related to impairment of udder defense. However, the effects of ketone body on some parameters of udder defense, such as SCC and release of inflammatory substances during IMI, need further investigation. In addition, mechanisms of impairment due to hyperketonemia have not yet been explored. In the future, it will be necessary to clarify the important mechanism relating to the impairment of the leukocyte function in NEB cows. We hypothesize that leukocytes from NEB cows have low amounts of enzymes for energy metabolism, such as glucose-6-phosphatase. In addition, the interference of the attachment between leukocytes and inflammatory

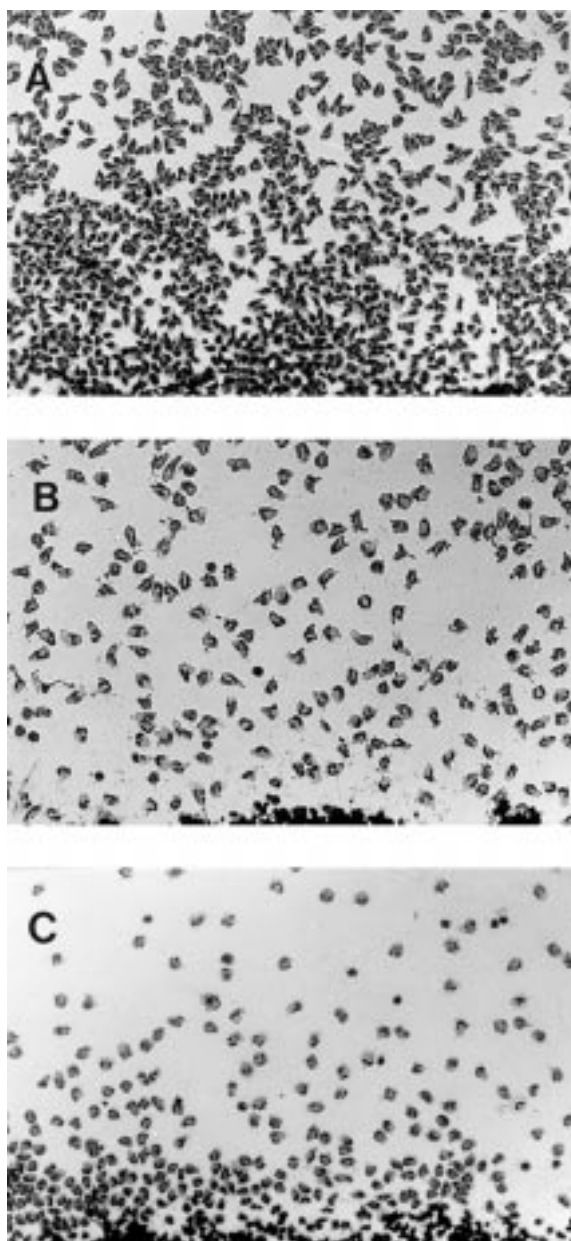


Figure 5. Concentration of migrating leukocytes and adhering properties of leukocytes to plastic culture dishes (magnification $100\times$). A. Leukocytes from a healthy cow in the standard media, as previously described [108], showed the highest concentration and adhered firmly to the culture dish. B. Leukocytes from a healthy cow in the standard media supplemented with BHB, AcAc (Acetoacetate), and Acetone at 1.0, 1.0, and 1.7 mM, respectively, showed less concentrations of leukocytes and had round, not flat, refractive features. C. Leukocytes from ketotic cows (BHB = 1.8 mM) cultured in the standard media showed less concentration of leukocytes and had round and not flat features.

mediators, which is necessary for chemotaxis process in vivo, may also be important mechanisms of impairment of the leukocyte function in the NEB cow. Subsequently, improvement of udder defense mechanisms in NEB cows might help reduce incidence of clinical mastitis in high-producing cows.

ACKNOWLEDGEMENTS

The author wishes to thank M. Nielsen, J.E. Kornalijnslipjer, T.N. van Werven, and V.P.M.G. Rutten for their helpful comments throughout the study. We also thank A.J.J.M. Daemen, D.E. Ott and D. Dijkhuizen for their technical assistance and P. Dobbelaar for the abstract in French.

REFERENCES

- [1] Aiello R.J., Armentano L.E., Gluconeogenesis in goat hepatocytes is affected by calcium, ammonia and other key metabolites but not primarily through cytosolic redox state. *Comp. Biochem. Physiol. B.* 88 (1987) 193-201.
- [2] Amaral-Phillips D.M., Mcgilliard A.D., Lindberg G.L., Veenhuizen J.J., Young J.W., Effects of decreased availability of glucose for dairy cows, *J. Dairy Sci.* 76 (1993) 752-761.
- [3] Anderson K.L., Smith L.A., DeGraves F.J., Hunt E., Fleming S.A., Polymorphonuclear neutrophil leukocyte function in clinical bovine patients and in cows with or without *Staphylococcus aureus* mastitis, *Vet. Res. Commun.* 16 (1992) 107-115.
- [4] Barber M.R., Yang T.J., Chemotactic activities in nonmastitic and mastitic mammary secretions: presence of interleukin-8 in mastitic but not nonmastitic secretions, *Clin. Diagn. Lab. Immunol.* 5 (1998) 82-86.
- [5] Bartlett P.C., Miller G.Y., Anderson C.R., Kirk J.H., Milk production and somatic cell count in Michigan dairy herds, *J. Dairy Sci.* 73 (1990) 2794-2800.
- [6] Bierman E.L., Glomset J.A., Disorders of lipid metabolism, in: Wilson J.D., Foster D.W. (Eds.), *Williams Textbook of Endocrinology*, WB Saunders company, Philadelphia, 1992, pp. 1367-1395.
- [7] Boudjellab N., Chan-Tang H.S., Li X., Zhao X., Interleukin 8 response by bovine mammary epithelial cells to lipopolysaccharide stimulation, *Am. J. Vet. Res.* 59 (1998) 1563-1567.
- [8] Boyum A., Wilk P., Gustavsson E., Veiby O.P., Reseland J., Haugen A.H., Optad P.K., The effect of strenuous exercise and calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. *Scand. J. Immunol.* 43 (1996) 228-235.
- [9] Breukink J., Wensing Th., Pathophysiology of the liver in high yielding dairy cows and its consequences for health and production, *Isr. J. Vet. Med.* 52 (1997) 66-72.
- [10] Burvenich C., Paape M.J., Hill A.W., Guidry A.J., Miller R.H., Heyneman R., Kremer W.D., Brand A., Role of the neutrophil leucocyte in the local and systemic reactions during experimentally induced *E. coli* mastitis in cows immediately after calving, *Vet. Q.* 16 (1994) 45-50.
- [11] Cai T.Q., Weston P.G., Lund L.A., Brodie B., McKenna D.J., Wagner W.C., Association between neutrophil functions and periparturient disorders in cows, *Am. J. Vet. Res.* 55 (1994) 934-943.
- [12] Carbone P., Sobreviela M., Jimenez D., Martinez C., Gonzalez de Aguero R., Pocovi M., Maternal leukocyte metabolism during pregnancy and puerperium and its relation to fetal growth, *Acta. Obstet. Gynecol. Scand.* 71 (1992) 266-272.
- [13] Colditz I.G., Watson D.L., Effect of immunisation on the early influx of neutrophils during staphylococcal mastitis in ewes, *Res. Vet. Sci.* 33 (1982) 146-151.
- [14] Cooray R., Björck L., Bactericidal activity of the bovine myeloperoxidase system against bacteria associated with mastitis, *Vet. Microbiol.* 46 (1995) 427-434.
- [15] Correa M.T., Erb H., Scarlett J., Path analysis for seven postpartum disorders of Holstein cows, *J. Dairy Sci.* 76 (1993) 1305-1312.
- [16] Craven N., Williams M.R., Defenses of the bovine mammary gland against infection and prospects for their enhancement, *Vet. Immunol. Immunopathol.* 10 (1985) 71-127.
- [17] Daley M.J., Coyle P.A., Williams T.J., Furda G., Dougherty R., Hayes P.W., *Staphylococcus aureus* mastitis: pathogenesis and treatment with bovine interleukin-1 beta and interleukin-2, *J. Dairy. Sci.* 74 (1991) 4413-4424.
- [18] Daley M.J., Oldham E.R., Williams T.J., Coyle P.A., Quantitative and qualitative properties of host polymorphonuclear cells during experimentally induced *Staphylococcus aureus* mastitis in cows, *Am. J. Vet. Res.* 52 (1991) 474-479.
- [19] Daley M., Williams T., Coyle P., Furda G., Dougherty R., Hayes P., Prevention and treatment of *Staphylococcus aureus* infections with recombinant cytokines, *Cytokine* 5 (1993) 276-284.
- [20] Daley M.J., Furda G., Dougherty R., Coyle P.A., Williams T.J., Johnston P., Potentiation of antibiotic therapy for bovine mastitis by recombinant bovine interleukin-2, *J. Dairy. Sci.* 75 (1992) 3330-3338.

- [21] Detilleux J.C., Grohn Y.T., Quaas R.L., Effects of clinical ketosis on test day milk yields in Finnish Ayrshire cattle, *J. Dairy Sci.* 77 (1994) 3316-3323.
- [22] Dosogne H., Burvenich C., van Werven T., Roets E., Noordhuizen-Stassen E.N., Goddeeris B., Increased surface expression of CD11b receptors on polymorphonuclear leukocytes is not sufficient to sustain phagocytosis during *Escherichia coli* mastitis in early postpartum dairy cows, *Vet. Immunol. Immunopathol.* 60 (1997) 47-59.
- [23] Drenth J.P., van Uum S.H., van Deuren M., Pesman G.J., van der Ven Jongekrijg J., van der Meer J.W., Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1 beta production, *J. Appl. Physiol.* 79 (1995) 1497-1503.
- [24] Erb H.N., Grohn Y.T., Epidemiology of metabolic disorders in the periparturient dairy cow, *J. Dairy Sci.* 71 (1988) 2557-2571.
- [25] Eshelman J.E., Eberhart R.J., Scholz R.W., Effects of cream on bactericidal and metabolic functions of bovine polymorphonuclear neutrophils, *Am. J. Vet. Res.* 42 (1981) 738-742.
- [26] Esslemont R.J., Peeler E.J., The scope for raising margins in dairy herds by improving fertility and health, *Br. Vet. J.* 149 (1993) 537-547.
- [27] Filar J., Kandefer-Szerszen M., Szuster-Ciesielska A., Rzeski W., Effects of cold treatment and ketosis induced by starvation on interferon production in leukocytes of lactating cows, *Dtsch. Tierärztl. Wochenschr.* 99 (1992) 210-213.
- [28] Fronk T.J., Schultz L.H., Hardie A.R., Effect of dry period overfeeding on subsequent metabolic disorders and performance of dairy cows, *J. Dairy Sci.* 63 (1980) 1080-1090.
- [29] Fukatsu A., Sato N., Shimizu H., Fifty-mile walking race suppresses neutrophil bactericidal function by inducing increases in cortisol and ketone bodies, *Life Sci.* 58 (1996) 2337-2343.
- [30] Gabriel, H., Müller H., Kettler K., Brechtel L., Urhausen A., Kindermann W., Increased phagocytic capacity of the blood., but decreased phagocytic activity per individual circulating neutrophil after an ultradistance run, *Eur. J. Appl. Physiol.* 71(1995) 281-284.
- [31] Garnsworthy P.C., Nutrition and lactation in the dairy cow, Butterworth, London, 1988.
- [32] Goff J.P., Horst R.L., Physiological changes at parturition and their relationship to metabolic disorders, *J. Dairy Sci.* 80 (1997) 1260-1268.
- [33] Gröhn Y.T., Erb H.N., McCulloch C.E., Salonen H., Epidemiology of metabolic disorders in dairy cattle and association among host characteristics, disease, and production, *J. Dairy Sci.* 72 (1989) 1876-1885.
- [34] Gröhn Y.T., Hertl J.A., Harman J.L., Effect of early lactation milk yield on reproductive disorders in dairy cows, *Am. J. Vet. Res.* 55 (1994) 1521-1528.
- [35] Grommers F.J., van de Geer D., van de Vliet H., Nijkamp F.P., Polymorphonuclear leukocyte function, relationship between induced migration into the bovine mammary gland and in vitro cell activity, *Vet. Immunol. Immunopathol.* 23 (1989) 75-83.
- [36] Guidry A.J., Oliver S.P., Squiggins K.E., Erbe E.F., Dowlen H.H., Hambleton C.N., Berning L.M., Effect of anticapsular antibodies on neutrophil phagocytosis of *Staphylococcus aureus*, *J. Dairy Sci.* 74 (1991) 3360-3369.
- [37] Hack V., Strobel G., Raw J.P., Weicker H., The effect of maximal exercise on the activity of neutrophil granulocytes in highly trained athletes in a moderate training period, *Eur. J. Appl. Physiol.* 65 (1992) 520-524.
- [38] Hack V., Strobel G., Weiss M., Weicker H., PMN cell counts and phagocytic activity of highly trained athletes depend on training period, *J. Appl. Physiol.* 77 (1994) 1731-1735.
- [39] Hallen S.C., Mamo W., Larsson I., Lindahl M., Bjork I., A periodate-sensitive anti-phagocytic surface structure, induced by growth in milk whey, on *Staphylococcus aureus* isolated from bovine mastitis, *Microb. Pathog.* 11 (1991) 211-220.
- [40] Harmon R.J., Physiology of mastitis and factors affecting somatic cell counts, *J. Dairy Sci.* 77 (1994) 2103-2112.
- [41] Herdt T.H., Fuel homeostasis in the ruminant, *Vet. Clin. North Am.* 4 (1988) 213.
- [42] Herdt T.H., Wensing T., Haagsman H.P., Golde L.M.G., Breukink H.J., Hepatic triacylglycerol synthesis during a period of fatty liver development in sheep, *J. Anim. Sci.* 66 (1988) 1997-2013.
- [43] Heuer C., Schukken Y.H., Dobbelaar P., Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds, *J. Dairy Sci.* 82 (1999) 295-304.
- [44] Heyneman R., Burvenich C., Vercauteren R., Interaction between the respiratory burst activity of neutrophil leukocytes and experimentally induced *Escherichia coli* mastitis in cows, *J. Dairy Sci.* 73 (1990) 985-994.
- [45] Hibbitt K.G., Neill D., Radford P., The effect of diet on the incidence of induced ketosis in the lactating dairy cow, *Res. Vet. Sci.* 10 (1969) 245-253.
- [46] Hill A.W., Factors influencing the outcome of *Escherichia coli* mastitis in the dairy cow, *Res. Vet. Sci.* 31 (1981) 107-112.
- [47] Hill A.W., Heneghan D.J., Williams M.R., The opsonic activity of bovine milk whey for the phagocytosis and killing by neutrophils of encapsulated and non-encapsulated *Escherichia coli*, *Vet. Microbiol.* 8 (1983) 293-300.

- [48] Hoeben D., Heyneman R., Burvenich C., Elevated levels of beta-hydroxybutyric acid in periparturient cows and in vitro effect on respiratory burst activity of bovine neutrophils, *Vet. Immunol. Immunopathol.* 58 (1997) 165-170.
- [49] Hoeben D., Burvenich C., Massart-Leen A.M., Lenjou M., Nijs G., van Bockstaele D., Beckers J.F., In vitro effect of ketone bodies, glucocorticosteroids and bovine pregnancy-associated glycoprotein on cultures of bone marrow progenitor cells of cows and calves, *Vet. Immunol. Immunopathol.* 68 (1999) 229-240.
- [50] Hogan J.S., Todhunter D.A., Tomita G.M., Smith K.L., Schoenberger P.S., Opsonic activity of bovine serum and mammary secretion after *Escherichia coli* J5 vaccination, *J. Dairy Sci.* 75 (1992) 72-77.
- [51] Kandefer-Szerszen M., Filar J., Szuster-Ciesielska A., Rzeski W., Suppression of interferon response of bovine leukocytes during clinical and subclinical ketosis in lactating cows, *Dtsch. Tierärztl. Wochenschr.* 99 (1992) 440-443.
- [52] Kehrli M.R. Jr., Shuster D.E., Factors affecting milk somatic cells and their role in health of the bovine mammary gland, *J. Dairy Sci.* 77 (1994) 619-627.
- [53] Kehrli M.E. Jr., Cullor J.S., Nickerson S.C., Immunobiology of hematopoietic colony-stimulating factors: potential application to disease prevention in the bovine, *J. Dairy Sci.* 74 (1991) 4399-4412.
- [54] Klucinski W., Degorski W., Miernik-Degorska E., Targowski S., Winnicka A., Effect of ketone bodies on the phagocytic activity of bovine milk macrophages and polymorphonuclear leukocytes, *J. Vet. Med. A* 35 (1988) 632-639.
- [55] Kremer W.D.J., Noordhuizen-Stassen E.N., Lohuis J.A.C.M., Host defense mechanisms and bovine coliform mastitis: a review, *Vet. Q.* 12 (1990) 103-113.
- [56] Kremer W.D.J., Burvenich C., Noordhuizen-Stassen E.N., Grommers F.J., Schukken Y.H., Heeringa R., Brand A., Severity of experimental *Escherichia coli* mastitis in ketonemic and nonketonemic dairy cows, *J. Dairy Sci.* 76 (1993) 3428-3436.
- [57] Kremer W.D.J., Noordhuizen-Stassen E.N., Grommers F.J., Daemen A.J.J.M., Henricks P.A.J., Burvenich C., Brand A., Preinfection chemotactic response of blood polymorphonuclear leukocytes to predict severity of *Escherichia coli* bovine mastitis, *J. Dairy Sci.* 76 (1993) 2514-2518.
- [58] Lean I.J., Bruss M.L., Baldwin R.L., Troutt H.F., Bovine Ketosis: A review, II. Biochemistry and prevention, *Veterinary Bulletin* 62 (1992) 1-14.
- [59] Lean I.J., Bruss M.L., Troutt H.F., Galland J.C., Farver T.B., Rostami J., Holmberg C.A., Weaver L.D., Bovine ketosis and somatotrophin: risk factors for ketosis and effects of ketosis on health and production, *Res. Vet. Sci.* 57 (1994) 200-209.
- [60] Leslie K.E., Dohoo I., Meek A.H., Somatic cell count in bovine milk, *Continuous Education Article* 5 (1984) S601-S612.
- [61] Lohuis J.A., Schukken Y.H., Henricks P.A.J., Preinfection functions of blood polymorphonuclear leukocytes and the outcome of experimental *Escherichia coli* mastitis in the cow, *J. Dairy Sci.* 73 (1990) 342-350.
- [62] Mamo W., Sandgren C.H., Lindahl M., Jonsson P., Induction of anti-phagocytic surface properties of *Staphylococcus aureus* from bovine mastitis by growth in milk whey, *J. Vet. Med. Ser. B.* 38 (1991) 401-410.
- [63] Marinetti G.V., Disorder of fatty acid metabolism, in: Marinetti G.V. (Ed.), *Disorders of lipid metabolism*, Plenum Press, New York 1990, pp. 31-47.
- [64] McMurray R.W., Bradsher R.W., Steele R.W., Pilkington N.S., Effect of prolonged modified fasting in obese persons on in vitro markers of immunity, lymphocyte function and serum effects on normal neutrophils, *Am. J. Med. Sci.* 299 (1990) 379-385.
- [65] Miller R.H., Paape M.J., Filep R., Flow cytometric analysis of neutrophils in cow's milk, *Am. J. Vet. Res.* 54 (1993) 1975-1979.
- [66] Mueller R., Carroll E.J., Panico L., Hemolytic complement titers and complement C3 levels in endotoxin-induced mastitis, *Am. J. Vet. Res.* 44 (1983) 1442-1445.
- [67] Nagahata H., Nochi H., Tamoto K., Yamashita K., Noda H., Kociba G.J., Characterization of functions of neutrophils from bone marrow of cattle with leukocytes adhesion deficiency, *Am. J. Vet. Res.* 56 (1995) 167-171.
- [68] Nakajima Y., Mikami O., Yoshioka M., Motoi Y., Ito T., Ishikawa Y., Fuse M., Nakano K., Yasukawa K., Elevated levels of tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) activities in the sera and milk of cows with naturally occurring coliform mastitis, *Res. Vet. Sci.* 62 (1997) 297-298.
- [69] Newsholme P., Curi R., Gordon S., Newsholme E.A., Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages, *Biochem. J.* 239 (1986) 121-125.
- [70] Newsholme P., Gordon S., Newsholme E.A., Rates of utilization and fates of glucose, glutamine, pyruvate, fatty acids and ketone bodies by mouse macrophages, *Biochem. J.* 242 (1987) 631-636.
- [71] Nickerson S.C., Effect of cytokines on bovine mammary gland immunity, *SAAS. Bull. Biochem. Biotechnol.* 4 (1991) 60-67.
- [72] Nickerson S.C., Pankey J.W., Neutrophil migration through teat end tissues of bovine mammary quarters experimentally challenged with

- Staphylococcus aureus*, J. Dairy Sci. 67 (1984) 826-834.
- [73] Nickerson S.C., Owens W.E., Rejman J.J., Oliver S.P., Effects of interleukin-1 and interleukin-2 on mammary gland leukocyte populations and histology during the early nonlactating period, Zentralbl. Veterinarmed. Reihe B 40 (1993) 621-633.
- [74] Oltenucu P.A., Ekesbo I., Epidemiological study of clinical mastitis in dairy cattle, Vet. Res. 25 (1994) 208-212.
- [75] Oresnik A., Effect of health and reproductive disorders on milk yield and fertility in dairy cows, Bovine Practitioner 29 (1995) 43-45.
- [76] Paape M.J., Guidry A.J., Effect of fat and casein on intracellular killing of *Staphylococcus aureus* by milk leukocytes, Proc. Soc. Exp. Biol. Med. 155 (1977) 588-593.
- [77] Paape M.J., Lilius E.M., Wiitanen P.A., Kontio M.P., Miller R.H., Intramammary defense against infections induced by *Escherichia coli* in cows, Am. J. Vet. Res. 57 (1996) 477-482.
- [78] Persson K., Larsson I., Hallen S.C., Effects of certain inflammatory mediators on bovine neutrophil migration in vivo and in vitro, Vet. Immunol. Immunopathol. 37 (1993) 99-112.
- [79] Persson-Waller K., Modulation of endotoxin-induced inflammation in the bovine teat using antagonists/inhibitors to leukotrienes, platelet activating factor and interleukin 1 beta, Vet. Immunol. Immunopathol. 57 (1997) 239-251.
- [80] Persson-Waller K., Colditz I.G., Seow H.F., Accumulation of leucocytes and cytokines in the lactating ovine udder during mastitis due to *Staphylococcus aureus* and *Escherichia coli*, Res. Vet. Sci. 63 (1997) 63-66.
- [81] Politis I., McBride B.W., Burton J.H., Zhao X., Turner J.D., Secretion of interleukin-1 by bovine milk macrophages., Am. J. Vet. Res. 52 (1991) 858-862.
- [82] Rainard P., Binding of bovine fibronectin to mastitis-causing *Streptococcus agalactiae* induces adherence to solid substrate but not phagocytosis by polymorphonuclear cells, Microb. Pathog. 14 (1993) 239-248.
- [83] Rainard P., Boulard C., Opsonization of *Streptococcus agalactiae* of bovine origin by complement and antibodies against group B polysaccharide, Infect. Immun. 60 (1992) 4801-4808.
- [84] Rainard P., Poutrel B., Deposition of complement components on *Streptococcus agalactiae* in bovine milk in the absence of inflammation, Infect. Immun. 63 (1995) 3422-3427.
- [85] Rainard P., Lautrou Y., Poutrel B., Ingestion and killing of *Streptococcus agalactiae* by bovine granulocytes in the presence of natural opsonins, Vet. Microbiol. 18 (1988) 41-50.
- [86] Rainard P., Sarradin P., Poutrel B., Phenotypic variability of X-protein expression by mastitis-causing *Streptococcus agalactiae* of serotype NT/X and opsonic activities of specific antibodies, Microb. Pathog. 16 (1994) 359-372.
- [87] Rainard P., Sarradin P., Paape M.J., Poutrel B., Quantification of C5a/C5a(desArg) in bovine plasma, serum and milk, Vet. Res. 29 (1998) 73-78.
- [88] Reddy P.G., Reddy D.N., Pruiett S.E., Daley M.J., Shirley J.E., Chengappa M.M., Blecha F., Interleukin 2 treatment of *Staphylococcus aureus* mastitis, Cytokine 4 (1992) 227-231.
- [89] Reid I.M., Roberts C.J., Treacher R.J., Williams L.A., Effect of body condition at calving on tissue mobilization development of fatty liver and blood chemistry of dairy cows, Anim. Prod. 43 (1986) 7-15.
- [90] Roets E., Burvenich C., Diez-Fraile A., Noordhuizen-Stassen E.N., Evaluation of the role of endotoxin and cortisol on modulation of CD18 adhesion receptors in cows with mastitis caused by *Escherichia coli*, Am. J. Vet. Res. 60 (1999) 534-540.
- [91] Rukkamsuk T., Wensing T., Geelen M.J., Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period, J. Dairy Sci. 81 (1998) 2904-2911.
- [92] Russell M.W., Brooker B.E., Reiter B., Inhibition of the bactericidal activity of bovine polymorphonuclear leucocytes and related systems by casein, Res. Vet. Sci. 20 (1976) 30-35.
- [93] Russell M.W., Brooker B.E., Reiter B., Electron microscopic observations of the interaction of casein micelles and milk fat globules with bovine polymorphonuclear leukocytes during the phagocytosis of *Staphylococci* in milk, J. Comp. Pathol. 87 (1977) 43-52.
- [94] Sanchez M.S., Ford C.W., Yancey R.J.J., Effect of tumor necrosis factor-alpha, interleukin-1 beta, and antibiotics on the killing of intracellular *Staphylococcus aureus*, J. Dairy Sci. 77 (1994) 1251-1258.
- [95] Sato S., Suzuki T., Okada K., Suppression of mitogenic response of bovine peripheral blood lymphocytes by ketone bodies, J. Vet. Med. Sci. 57 (1995) 183-185.
- [96] Schukken Y.H., Mallard B.A., Dekkers J.C.M., Leslie K.E., Stear M.J. Genetic impact on the risk of intramammary infection following *Staphylococcus aureus* challenge, J. Dairy Sci. 77 (1994) 639-647.
- [97] Schukken Y.H., Leslie K.E., Barnum D.A., Mallard B.A., Lumsden J.H., Dick P.C., Vessie G.H., Kehrl M.E. Experimental *Staphylococcus aureus* intramammary challenge in late lactation dairy cows: quarter and cow effects determining the probability of infection, J. Dairy Sci. 82 (1999) 2393-2401.
- [98] Schultze W.D., Paape M.J., Effect on outcome of intramammary challenge exposure with *Staphylococcus aureus* of somatic cell concen-

- tration and presence of an intramammary device, *Am. J. Vet. Res.* 45 (1984) 420-423.
- [99] Serlenga E., Garofalo A.R., De Pergola G., Ventura M.T., Tortorella C., Antonaci S., Polymorphonuclear cell-mediated phagocytosis and superoxide anion release in insulin-dependent diabetes mellitus, *Cytobios* 74 (1993) 189-195.
- [100] Shuster D.E., Kehrl M.E.J., Administration of recombinant human interleukin 1 receptor antagonist during endotoxin-induced mastitis in cows, *Am. J. Vet. Res.* 56 (1995) 313-320.
- [101] Shuster D.E., Kehrl M.E.J., Stevens M.G., Cytokine production during endotoxin-induced mastitis in lactating dairy cows, *Am. J. Vet. Res.* 54 (1993) 80-85.
- [102] Shuster D.E., Lee E.K., Kehrl M.E.Jr., Bacterial growth, inflammatory cytokine production, and neutrophil recruitment during coliform mastitis in cows within ten days after calving, compared with cows at midlactation, *Am. J. Vet. Res.* 57 (1996) 1569-1575.
- [103] Shuster D.E., Kehrl M.E., Rainard P., Paape M., Complement fragment C5a and inflammatory cytokines in neutrophil recruitment during intramammary infection with *Escherichia coli*, *Infect. Immun.* 65 (1997) 3286-3292.
- [104] Simianer H., Solbu H., Schaeffer L.R., Estimated genetic correlation between disease and yield traits in dairy cattle, *J. Dairy Sci.* 74 (1991) 4358-4365.
- [105] Smits E., Burvenich C., Guidry A.J., Roets E., In vitro expression of adhesion receptors and diapedesis by polymorphonuclear neutrophils during experimentally induced *Streptococcus uberis* mastitis, *Infect. Immun.* 66 (1998) 2529-2534.
- [106] Sordillo L.M., Shafer-Weaver K., DeRosa D., Immunobiology of the mammary gland, *J. Dairy Sci.* 80 (1997) 1851-1865.
- [107] Suriyasathaporn W., Daemen A.J.J.M., Schukken Y.H., Effect of serum β -hydroxybutyrate levels on in vitro chemotaxis of bovine leukocytes, 10th international conference of production diseases in farm animals, 24-28 August 1998, Utrecht, The Netherlands, p. 266.
- [108] Suriyasathaporn W., Daemen A.J., Noordhuizen-Stassen E.N., Dieleman S.J., Nielen M., Schukken Y.H., Beta-hydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes, *Vet. Immunol. Immunopathol.* 68 (1999) 177-186.
- [109] Suriyasathaporn W., Brand A., Nielen M., Schukken Y.H., Low somatic cell count is a risk factors for clinical mastitis, *J. Dairy Sci.* (in press).
- [110] Syvajarvi J., Saloniemi H., Grohn Y.T., An epidemiological and genetic study on registered diseases in Finnish Ayrshire cattle IV Clinical mastitis, *Acta Vet. Scand.* 27 (1986) 223-234.
- [111] Targowski S.P., Klucinski W., Reduction in mitogenic response of bovine lymphocytes by ketone bodies, *Am. J. Vet. Res.* 44 (1983) 828-830.
- [112] Uribe H.A., Kennedy B.W., Martin S.W., Kelson D.F., Genetic parameters for common health disorders of Holstein cows, *J. Dairy Sci.* 78 (1995) 421-430.
- [113] van den Top A.M., Geelen M.J., Wensing T., Wentink G.H., van't Klooster A.T., Beynen A.C., Higher postpartum hepatic triacylglycerol concentrations in dairy cows with free rather than restricted access to feed during the dry period are associated with lower activities of hepatic glycerolphosphate acyltransferase, *J. Nutr.* 126 (1996) 76-85.
- [114] van Werven T., The role of leukocytes in bovine *Escherichia coli* mastitis, Ph.D. thesis, University of Utrecht, The Netherlands, 1999.
- [115] van Werven T., Noordhuizen-Stassen E.N., Daemen A.J., Schukken Y.H., Brand A., Burvenich C., Preinfection in vitro chemotaxis, phagocytosis, oxidative burst, expression of CD11/CD18 receptors and their predictive capacity on the outcome of mastitis induced in dairy cows with *Escherichia coli*, *J. Dairy Sci.* 80 (1997) 67-74.
- [116] Wentink G.H., Rutten V.P., van den Ingh T.S., Hoek A., Muller K.E., Wensing T., Impaired specific immunoreactivity in cows with hepatic lipidosis, *Vet. Immunol. Immunopathol.* 56 (1997) 77-83.
- [117] Wilson R.M., Reeves W.G., Neutrophil phagocytosis and killing in insulin-dependent diabetes, *Clin. Exp. Immunol.* 63 (1986) 478-484.
- [118] Zecconi A., Bronzo V., Piccinini R., Spreafico G., Ruffo G., Phagocytic activity of bovine polymorphonuclear neutrophil leucocytes, *J. Dairy Res.* 61 (1994) 271-279.