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ENERGY IMBALANCE RELATED PREDISPOSITION TO MASTITIS IN GROUP-FED HIGH-PRODUCING POSTPARTUM DAIRY COWS

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The energy imbalance related predisposition to mastitis was studied in group-fed postpartum dairy cows (n = 333) kept in 4 large-scale units and producing milk of low somatic cell count (SCC). Blood samples were taken on Days 1–3 after calving for assaying some metabolites and hormones related to the negative energy balance (NEB). If mastitis was diagnosed later, aseptic milk samples were taken to identify the pathogens. Considering pathogen types [contagious pathogens: *Staphylococcus* (*S.*) *aureus*, *Gram-positive* (GP) *environmental pathogens*, and *Gram-negative* (GN) *environmental pathogens* + mastitis with *no detectable pathogens* (NDP)] separately, stepwise logistic regression was used to analyse the relation between the potential prognostic value of hormones and metabolites and mastitis outbreak. Only the elevated (≥ 1.00 mmol/l) serum β-hydroxybutyrate (BHB) levels predisposed the cows to mastitis in the subsequent 4 weeks. This prognostic value of BHB was significant only in GN + NDP mastitis and in cases caused by GP environmental pathogens, but not in *S. aureus* mastitis (odds ratio: 5.333, 3.600 and 1.333, respectively).

Key words: Mastitis, ketosis, ovary, dairy cow, postpartum period

Several epidemiological studies have clearly demonstrated interrelations between the decompensated forms (fatty liver, ketosis) of postpartum (pp) negative energy balance (NEB) and the diminished capacity of intramammary and intrauterine defence mechanisms resulting in increased incidence of clinical mastitis and metritis (Schukken et al., 1988; Valde et al., 1997; Washburn et al., 2002). This increased incidence of mastitis and bacterial complications in uterine

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involution seems to be associated mainly with the periparturient impairments in leukocyte migration and function resulting from elevated levels of β-hydroxybutyrate (BHB) and/or non-esterified fatty acids (NEFA), as well as from other metabolic changes in the early weeks of lactation (Klucinski et al., 1988*a, b*; Kremer et al., 1993*b*; Cai et al., 1994; Sartorelli et al., 1999; Suriyasathaporn et al., 1999; Sartorelli et al., 2000; Zerbe et al., 2000; Suriyasathaporn et al., 2000; Kimura et al., 2002).

Despite their expected clinical relevance, however, we can only hardly find well-controlled prospective field trials studying the hyperketonaemia-related predisposition for mastitis in commercial dairy herds. In the mid- to late 1990s series of studies were carried out in some large-scale dairy units in Hungary in order to determine whether pp hyperketonaemia was a real predisposing factor for mastitis. In the current report we wish to present these findings.

Materials and methods

Farm conditions and animals

The experiment was conducted in 4 commercial large-scale dairy herds with about 500 to 1850 Holstein Friesian cows and their crosses in each, yielding about 7000–8300 kg fat-corrected milk per cow in average. Each farm had its mastitis control program including the regular, once-a-month checking of somatic cell count (SCC) in individual bulk milk samples. All herds had been producing extra-quality milk of low (< 400,000/ml) SCC for many years. In all of these herds the cows were kept in a free housing system with no possibility for pasturing and individual feeding, in groups of 70 to 100 animals formed in accordance with their stage of lactation and their monthly checked actual daily milk yield. In the sheds straw-bedded resting place was provided for the animals. Dry cows were housed separately and they calved in maternity units in groups of 3–4 animals. Separated groups were formed for fresh-milking heifers and cows. All the cows were milked three times (about > 30 kg milk/day) or twice (about < 30 kg milk/day) a day in a milking house with BouMatic type machinery (Herds A, B and C), or twice only in a milking house equipped with Alfa-Laval technology (Herd D). The clinical condition of the udder was checked at all milking procedures. If any mastitis-related signs (Table 1) were seen, the cow was separated and treated with antimicrobials. The daily ration was made up from ensilaged maize and alfalfa products, alfalfa and grass hay and cereals completed with vitamins and minerals, in accordance with the NRC (1989) recommendations.

Table 1

Recording and scoring the clinical signs of mastitis (after Pyörälä and Syvajarvi, 1987)

	Score 1	Score 2	Score 3
Systemic signs	None	Rectal temperature: ≤ 40.5 °C and/or slight anorexia and depression	Rectal temperature: > 40.5 °C and/or severe anorexia and depression, or recumbency
Local signs	None	Moderate swelling + tenderness of the affected quarter(s)	Severe swelling, firmness, the quarter very sore to touch
Milk appearance	Normal	Slightly watery, discoloured, and/or clots and flakes	Consistency serum-like, pus-like and/or bloody

All of the ≥ 2 parity cows which (1) yielded low (< 400,000/ml) SCC individual bulk milk continuously and were free from the clinical signs of chronic recurrent mastitis in their previous lactation (furthermore in Herds B and C: did not show positivity in any of their udder quarters by the California Mastitis Test in the first 1–3 days pp), and (2) calved within the pre-selected periods of the study (Herds A and B: October to November, 1996; Herd C: May to June, 1997; Herd D: July to August, 1998 and 1999) were involved in the trial, unless they needed veterinary intervention at calving, calved twins, and/or showed clinical symptoms of parturient paresis, hepatic injuries or mastitis before the onset of the sampling procedure.

Design of sampling. Clinical examination and scoring of mastitis

The farms were visited twice a week and all the cows (n = 335) that had calved 1 to 3 days before and met the above criteria were enrolled in the study. A blood sample was taken from each of them immediately for assaying certain hormones, metabolites and enzymes known to be related to energy metabolism and liver function. Also the onset of ovarian activity of cows was monitored by individual progesterone profiles, but the introduction of this procedure and their results are not the subject of the current report.

When later clinical symptoms of mastitis (Table 1) were observed, the affected cow was separated immediately. The date of outbreak and the severity of symptoms were recorded and scored on a scale from 1 (no changes) to 3 (severe reaction) as described by Pyörälä and Syvajarvi (1987) (Table 1), and aseptic milk samples were taken to isolate and identify the mastitis pathogens. As treatment, commercially available cephalosporin-containing preparations (235 mg of

cephacetrile sodium[1] or 250 mg of cefoperazone[2]) were administered locally once a day for 1–4 days. When more than one quarters were affected simultaneously, or after a temporary clinical recovery symptoms of mastitis were observed again, the cow was evaluated only if the same pathogen was isolated from the different quarters or occurrences.

Technical aspects of sampling. Laboratory procedures

To assay the basal concentrations of cortisol, 3,3',5-triiodothyronine (T_3), thyroxine (T_4), insulin and insulin-like growth factor-I (IGF-I), furthermore the circulating levels of glucose, acetoacetic acid (AcAc), BHB, NEFA, total cholesterol (TCh) and urea, as well as the activity of aspartate aminotransferase (AST) enzyme, blood samples were collected on Days 1 to 3 after calving. All these samples were taken from the jugular vein into heparinised and sodium fluoride containing tubes (the latter one for glucose determination) about 60 min after the morning milking (e.g. just before the morning feeding). The blood samples were centrifuged within 30 min, and the plasma was kept at +4 °C and assayed within 24 h (for glucose), or was stored frozen at –20 °C until the other hormone and metabolite determinations (Tables 2 and 3).

The aseptic milk samples taken for bacteriology were stored at –20 °C and transported to the lab twice a week. Mastitis pathogens were isolated according to Honkanen-Buzalski and Seuna (1995). From each sample 0.01 ml milk was streaked onto the surface of one-fourth plate of 90 mm Columbia agar[3] containing 5% sheep blood and 0.01% esculin. All the plates were incubated at 37 °C, and were evaluated after 14 to 16 h and again following an additional 24 h. The colonies were tentatively identified according to their morphology, pigment production, Gram staining, catalase test and the type of haemolysis produced. The pure cultures were identified based on the recommendations of Quinn et al. (1994) and Honkanen-Buzalski and Seuna (1995). If no bacteria were isolated, the case was considered as *mastitis caused by not detectable pathogens* (thereafter: NDP mastitis).

Data evaluation

In the current report data of cows with and without clinical mastitis in the first 28 days after calving are compared.

In accordance with the literature (Bruss, 1997) 1.00 mmol/l of BHB level was estimated as a borderline between hyperketonaemic (≥ 1.00 mmol/l) and

[1]Vetimast®, Novartis Animal Health, Basle, Switzerland
[2]Pathozone®, Pfizer Animal Health, Exton, PA, USA
[3]Merck, KgaA, Darmstadt, Germany

normoketonaemic (< 1.00 mmol/l) conditions. The diagnosis of acute putrid endometritis (APE) was based on the presence of malodorous, reddish-brown, watery vaginal discharge with or without toxic general symptoms (anorexia, lethargy, decreased rumen motility, elevated temperature) on Days 6–12 after calving.

The results were subjected to chi-squared test (distributions), Student's *t*-test (pair-wise comparison of group means) or one-way analysis of variance (ANOVA) (comparison of ≥ 3 groups). The statistical significance of differences between means of ≥ 3 groups was estimated by calculating the least significant difference (LSD) (Kleinbaum and Kupper, 1978; Juvancz and Paksy, 1981). For estimation of prognostic value attributable to circulating hormone and metabolite levels being above/below a certain threshold the odds ratio was calculated. Considering pathogen *types 1* (contagious pathogens: *S. aureus*), *2* [*Gram-positive* (GP) *environmental pathogens*], and 3 [*Gram-negative* (GN) *environmental pathogens* + NDP mastitis; for further details see also Table 4] separately, stepwise logistic regression was used to analyse the relation between potential prognostic factors (hormone and metabolite levels) and mastitis outbreak. The cases of *mastitis combined with APE* were evaluated separately in the same way, but (due to the limited number of cases) regardless of the type of the isolated mastitis pathogens. Analyses were done using the statistical package SPSS for Windows 8.0.

Results

One hundred and forty-six of the 333 cows had mastitis in the first 28 days after calving (identified pathogens: Table 4). Eleven of them died or were emergency slaughtered, and in further 49 of them also APE was diagnosed on Days 6–12 after calving. In the affected quarters of these 49 cows mainly environmental pathogens were identified (n = 32), or no pathogens were detected (NDP mastitis, n = 12), while contagious pathogens represented by *S. aureus* were found only in 5 of them. Only one of the 189 non-mastitic cows was affected by APE.

The cows with GN or NDP mastitis usually showed more severe clinical symptoms than those with GP mastitis (Table 5). In the severity of symptoms, however, no significant differences were found between the cases observed in the first vs. the second 2-week-long periods.

On Days 1–3 after calving the cows affected by mastitis in the subsequent 4 weeks (as a single disease or in combination with APE) were characterised by significantly more elevated AcAc, BHB and NEFA, and lower IGF-I, T_4 and T_3 levels than their non-mastitic herd-mates (Table 6). No similar mastitis-related differences were detected, however, in AST activity and in glucose, TCh, urea, cortisol and insulin concentrations. These metabolic and endocrine differences derived mainly from data of cows infected with GP [coagulase-negative staphy-

lococci, *Streptococcus* (*Str.*) *dysgalactiae, Str. uberis*, and other *Str.*] and GN (*E. coli* and *Klebsiella* spp.) environmental pathogens, plus from cases of NDP mastitis, rather than from those with *S. aureus* mastitis (Table 7). In the first 4 weeks of lactation *S. aureus* mastitis occurred almost at the same rate in the hyperketonaemic as in the normoketonaemic cows. However, the incidence of mastitis caused by GP and GN environmental pathogens plus of those with NDP was significantly higher among the hyperketonaemic than normoketonaemic individuals (Fig. 1). In mastitis caused by environmental pathogens clear hyperketonaemia-related predisposition was verified also by calculating the odds ratio (Table 8). The hyperketonaemia-based prediction was more pronounced in GN plus NDP than in GP mastitis, and it was quite obvious also in cows with mastitis plus APE. However, no significant forecasting value was attributable to BHB elevation in *S. aureus* mastitis, and despite the differences in Day 1–3 mean concentrations the odds ratio could not confirm the predictive value of any of the other endocrine or metabolic parameters either (no details are given). Retained fetal membrane, however, was a more important predisposing factor for mastitis plus APE than hyperketonaemia (Table 8). The severity of clinical symptoms were not influenced by hyperketonaemia (data not shown).

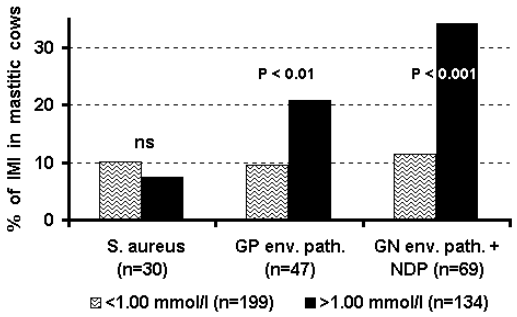

Fig. 1. The β-hydroxybutyrate-related distribution of mastitic cases caused by *S. aureus*, Gram-positive and Gram-negative environmental pathogens plus those with no detectable pathogens (NDP) in the first 4 weeks of lactation in Experiment 1. (Note: 5, 7 and 12, as well as 0, 11 and 14 of these cases were affected also by acute putrid endometritis in the normoketonaemic and hyperketonaemic cows, respectively. IMI: intramammary infection)

Discussion

In the current trial we investigate the clinical relevance of metabolic predisposition to mastitis in group-fed pp dairy cows. Concerning the metabolic aspects the supposed predictive value of elevated ketone (BHB, AcAc) levels and other metabolic and endocrine parameters indicating the existence and degree of NEB was studied in the earliest pp days. The chosen cut-off level of 1.00 mmol/l BHB

in plasma between hyperketonaemic (≥ 1.00 mmol/l) and normoketonaemic (< 1.00 mmol/l) conditions was in agreement with the literature (Bruss, 1997), and was accepted also by others (Sartorelli et al., 2000) in similar trials. In model studies plasma levels of BHB < 0.80, 0.8–1.60 and ≥ 1.60 mmol/l were considered as low, medium and high concentrations, respectively (Suriyasathaporn et al., 1999). The same border in plasma AcAc is about 0.35 mmol/l (Bruss, 1997; Sartorelli et al., 2000). However, due to its analytical uncertainties AcAc is considered usually as a less expensive, but also less reliable parameter (Bruss, 1997).

The endocrine, metabolic and immune responses of high-yielding dairy cows to pp NEB (Suriyasathaporn et al., 2000) are known to have clear age-related differences. To avoid this source of variance only 2nd parity and older cows were involved.

This study was intended to extend to almost all sorts of intramammary infections including the predominant contagious microbe of *S. aureus*, as well as the most important GP and GN environmental pathogens. However, in the early weeks of lactation GN bacteria may be the predominant mastitis pathogens in herds producing milk of low (< 150,000–250,000/ml) SCC (Hogan et al., 1989; Schukken et al., 1989; Green et al., 1996; Miltenburg et al., 1996; Beaudeau et al., 2002; Peeler et al., 2002), as well as their prevalence is known to increase also in the spring and summer months (Sandholm and Pyörälä, 1995; Zerocelli and Piccinini, 2002), mainly in cows heavily and persistently contaminated with faeces (Ward et al., 2002). So this trial was carried out in large-scale dairy herds producing low SCC milk for many years, individuals with healthy udder were involved, and most of the sampling series were conducted in seasons supposed to provide the most suitable environmental conditions for GN pathogens. Due to these restrictions, however, these data cannot be suitable for the estimation of the average incidence of the main mastitis pathogens in these herds.

In all of our previous studies the endocrine and metabolic alterations seen in cows with GN mastitis were very close to our corresponding findings in NDP mastitis (Jánosi, 2002). In accordance with the literature (Carroll et al., 1973; Barrow and Hill, 1989; Honkanen-Buzalski, 1995; Sandholm and Pyörälä, 1995; Fang and Pyörälä, 1996) we think that at the beginning the overwhelming part of these inflammatory processes were induced by intracisternal GN (mainly *E. coli*) infections, but before sampling the original pathogens were eliminated by the self-defence mechanisms of the udder. So due to this supposed endotoxin-derived character of this process in the current trial we pooled the data of cows with GN and NDP mastitis in order to reach the sufficient number of cows for reliable statistical evaluation. The other important prerequisite (Honkanen-Buzalski, 1995; Pyörälä, 1995) allowing us this pooling was that cows with chronic recurrent mastitis in their previous lactation were not included in this study.

Table 2

Endocrine assay procedures

Hormone	Technique	Kit/Literature	Sensitivity	Intraassay CV	Interassay CV
Hormones from blood (heparinised plasma) samples*					
Cortisol	^{3}H-RIA	Csernus (1982)	0.27 nmol/l	3.2–8.7%	≤ 10.3%
Thyroxine (T_4)	^{125}I-RIA	^{125}I-T_4-Spec RIA MIS kit[1]	0.46 nmol/l	6.6–8.5%	≤ 7.7%
3,3',5-triiodothyronine (T_3)	^{125}I-RIA	^{125}I-T_3 RIA MIS kit[1]	0.18 nmol/l	6.2–8.8%	≤ 6.7%
Insulin	^{125}I-RIA	Samples from Herds A–B: ^{125}I-Insulin RIA PEG kit[2]	1.94 μIU/ml	7.7–10.2%	≤ 12.3%
		Samples from Herds C–D: ^{125}I-Insulin RIA CT kit[3]	1.88 μIU/ml	5.5–8.4%	≤ 8.8%
Insulin-like growth factor-I (IGF-I)	After ethanol-acetic acid extraction, with ^{125}I-RIA	Samples from Herds A–B: Nap et al. (1993)[4]	0.25 nmol/l	3.6–6.5%	≤ 12.5%
		Samples from Herds C–D: Nikolic et al. (2001)	0.24 nmol/l	3.0–6.0%	≤ 12.0%

[1]Institute of Isotopes Co. Ltd. (Budapest, Hungary); [2]With the ^{125}I-Insulin RIA PEG kit of the Institute of Isotopes Co. Ltd. (Budapest, Hungary); [3]With the ^{125}I-Insulin RIA CT kit of CIS Bio International Ltd. (Gif-Sur-Yvette, France); [4]The method was slightly modified and validated for assaying IGF-I in bovine plasma (Dieleman, unpublished); *All methods were validated for assaying bovine plasma systems previously. The binding pattern of serially diluted bovine samples was parallel to that of the standard curves, and the recovery of added hormones from bovine plasma varied between 94 and 106%

Table 3: Colorimetric assay procedures used for determination of metabolites in plasma samples

Parameter	Technique	Kit / Literature
Aspartate aminotransferase (AST)	IFCC, UV method*	Reanal Ltd., Budapest, Hungary (Kit 7249)
Glucose	Enzymatic (GOD-POD) reaction*	Diagnosticum Ltd., Budapest, Hungary (Kit 40841)
Acetoacetate (AcAc)	Salicylaldehyde reaction	Walker (1954)
β-hydroxybutyrate (BHB)	β-hydroxybutyrate dehydrogenase reaction*	Sigma Diagnostics Co., (Kit 310-A)
Non-esterified fatty acid (NEFA)	Extraction of their colored soaps	Noma et al. (1973)
Total cholesterol (TCh)	Enzymatic (CHOD-PAP) reaction*	Diagnosticum Ltd., Budapest, Hungary (Kit 40121)
Urea	Enzymatic (urease) reaction	Reanal Ltd., Budapest, Hungary (Kit 16571)

*Measured on an automated clinicochemical analyser (Eppendorf ACP 5040)

Table 4: Isolated and identified mastitis pathogens

	Mastitis on Day ≤ 28			
	Mastitis with APE	Mastitis as a single disease		
		Lost♦	Days 1–14	Days 15–28
Gram-positive (GP) contagious pathogens:				
S. aureus	5	2	17	6
Gram-positive (GP) environmental pathogens				
Coagulase-negative staphylococci	1		7	1
Str. dysgalactiae	4		4	1
Str. uberis	9	1	8	6
Other (faecal) streptococci	4		1	
Gram-negative (GN) environmental pathogens				
E. coli	14	4	14	8
Klebsiella				
No detectable pathogen (NDP)	12	4	8	5
All	49	11	59	27

APE: acute putrid endometritis diagnosed on Days 6–12; ♦Lost: number of cows that died or were emergency slaughtered due to mastitis

Table 5

Severity of clinical symptoms in mastitis caused by various pathogens as a single disease in the first and second 2-week periods after calving

			Rectal temperature, °C	Score of			
				systemic signs	local signs	milk appearance	altogether
Mastitis on Days 1–14	GP (n = 40)	x ± SD	39.7 ± 0.6	1.4 ± 0.7	1.8 ± 0.7	2.1 ± 0.3	5.2 ± 1.4
	GN + NDP (n = 30)	x ± SD	40.7 ± 0.8	2.7 ± 0.5	2.4 ± 0.6	2.4 ± 0.5	7.4 ± 1.3
	P <		0.001	0.001	0.001	0.05	0.001
Mastitis on Days 15–28	GP (n = 14)	x ± SD	39.5 ± 0.7	1.6 ± 0.6	1.8 ± 0.7	2.1 ± 0.4	5.5 ± 1.5
	GN + NDP (n = 13)	x ± SD	40.4 ± 0.5	2.5 ± 0.7	2.5 ± 0.5	2.4 ± 0.5	7.3 ± 1.3
	P <		0.001	0.01	0.01	ns	0.01

GN: Gram-negative; GP: Gram-positive; NDP mastitis: mastitis with no detectable pathogens; ns: non-significant

Table 6

The Day 1–3 levels of certain metabolites and hormones in plasma of non-mastitic cows and of those with mastitis on Days 1–14 and Days 15–28, as a single disease, or in combination with acute putrid endometritis (APE)

		AcAc (mmol/l)	BHB (mmol/l)	NEFA (mmol/l)	IGF-I (nmol/l)	T_4 (nmol/l)	T_3 (nmol/l)
Non-mastitic (n = 189)	x ± SD	0.132 ± 0.086	0.89 ± 0.56	0.358 ± 0.217	4.72 ± 1.77	28.84 ± 9.35	1.18 ± 0.29
Mastitis, D 1–14 (n = 70)	x ± SD	0.150 ± 0.114	1.12 ± 0.58	0.447 ± 0.221	3.99 ± 1.57	25.54 ± 6.02	1.10 ± 0.18
Mastitis, D 15–29 (n = 27)	x ± SD	0.273 ± 0.394	1.18 ± 0.50	0.454 ± 0.261	4.02 ± 1.59	26.68 ± 5.08	1.10 ± 0.19
Mastitis + APE (n = 49)	x ± SD	0.157 ± 0.124	1.15 ± 0.68	0.436 ± 0.219	4.12 ± 1.50	25.69 ± 6.48	1.11 ± 0.22
F =		7.33	5.22	4.21	4.56	4.01	2.52
$LSD_{(P < 0.05)}$ =		0.054	0.21	0.082	0.62	2.99	0.09

Table 7

The Day 1–3 levels of certain metabolites and hormones in plasma of mastitic cows affected by contagious pathogens (*S. aureus*) vs. environmental pathogens plus NDP on Days 1–14 [including the data of those with acute putrid endometritis (APE)]

		AcAc (mmol/l)	BHB (mmol/l)	IGF-I (nmol/l)	T_4 (nmol/l)	T_3 (nmol/l)
Mastitis caused by *S. aureus* (n = 24)	x ± SD	0.094 ± 0.076	0.80 ± 0.34	4.52 ± 1.58	27.17 ± 7.25	1.22 ± 0.22
Mastitis caused by GP + GN environmental pathogens + NDP (with or without APE) (n = 95)	x ± SD	0.168 ± 0.122	1.21 ± 0.65	3.80 ± 1.55	25.19 ± 5.86	1.06 ± 0.16
P <		0.001	0.001	(0.1)	(0.1)	0.01

Table 8

The predictive value of retained fetal membrane and Day-1–3 hyperketonaemia for occurrence of clinical mastitis caused by various pathogens in the first 4 weeks of lactation (as a single disease, or in combination with acute putrid endometritis)

	Odds value	95% confidence interval	
		Lower	Upper
The predictive value of day-1–3 hyperketonaemia (e.g. BHB level: ≥ 1.00 mmol/l) for			
Mastitis as a single disease, caused by			
1) *S. aureus*	1.333 (ns)	0.585	3.041
2) GP environmental pathogens	3.600 (P < 0.01)	1.857	6.977
3) GN environmental pathogens + NDP	5.333 (P < 0.001)	2.941	9.670
Mastitis* combined with acute putrid endometritis	1.63 (P = 0.102)	0.84	3.19
The predictive value of retained fetal membrane for mastitis* combined with acute putrid endometritis	16.20 (P < 0.001)	7.21	36.42

GN: Gram-negative; GP: Gram-positive; NDP mastitis: mastitis with no detectable pathogens; *Regardless of the isolated pathogens

In epidemiological studies the NEB and its decompensation (hepatic lipidosis and/or ketosis) were associated with an increased risk of clinical mastitis, as well as of bacterial complications in uterine involution, such as APE (Markusfeld, 1985; Erb and Gröhn, 1988; Schukken et al., 1988; Correa et al., 1993; Oltanecu and Ekesbo, 1994; Valde et al., 1997; Washburn et al., 2002). Also the course of an experimentally induced *E. coli* mastitis proved to be more severe in ketotic than in non-ketotic individuals (Kremer et al., 1993*a*). This increased incidence and more severe course of these bacterial diseases can be explained by impairments in the antimicrobial self-defence mechanisms, mainly in polymorphonuclear granulocyte and monocyte migration and function (Cai et al., 1994; Suriyasathaporn et al., 1999; Kimura et al., 2002).

In the current experiment we collected data on the predictive value of certain plasma metabolites and metabolic hormones. For this purpose, samples were taken on Days 1–3 after calving and several parameters informing us on the current stage of NEB and/or liver function were determined. The cows affected by mastitis as a single disease or in combination with APE some days later in the early puerperium showed more elevated AcAc, BHB and NEFA, and lower IGF-I, T_4 and T_3 levels than those which remained healthy during the first 4 weeks after calving. This tendency related to a more severe form of energy imbalance (Haraszti et al., 1982; Kunz et al., 1985; Ronge et al., 1988; Giger et al., 1997; Blum et al., 2000), and derived mainly from parameters of mastitic cows infected with GP and GN environmental pathogens or affected by NDP mastitis. In addition, just after calving the data of cows with *S. aureus* intramammary infection were very close to their healthy herd-mates. By calculating the odds ratio we could attribute significant predictive value only to the elevation of BHB, but not to any other NEB-related changes in circulating levels of hormones and metabolites. This predictive value was highly significant for GN microbes, slightly less obvious for GP environmental pathogens and questionable, if any, for contagious pathogens. Also a certain BHB-associated susceptibility was observed to mastitis combined with APE (although this hyperketonaemia-related predisposition was less obvious than the effect of retained fetal membrane). Due to the limited number of these cases, however, we could not estimate the degree of BHB-related predisposition for separated groups of pathogens. Based on these findings we suppose that in the early weeks of lactation the hyperketonaemia, rather than the NEB itself, predisposes the cow to mastitis. The pathogen-dependent character of this predisposition may be explained by differences in mechanisms by which the udder can prevent and/or eliminate the various forms of microbial infections. Hyperketonaemia may depress mainly those components of the antimicrobial self-defence which are responsible for the destruction of invading environmental pathogens stuffed into the cisternal system and/or into the lactiferous ducts. *S. aureus*, however, is able to actively colonise the teat apex, first of all in cases of epithelial injury. Following their adhesion these bacteria can adapt to the milk

environment, forming a mucopolysaccharide capsule to avoid phagocytosis. After penetration through the teat barrier into the cistern the invading pathogen is able to adhere to milk fat globules and can float upwards in the udder (Pyörälä, 1995). In accordance with the literature (Pyörälä, 1995; Zadoks et al., 2001) we think that several other factors [e.g. some kind of lowered resistance: change of environmental temperature, virus infection (bovine herpesvirus 4 and others), recovery from a preceding mastitis, other infected quarter(s) of the same cow, extremely callused teat ends, and epithelial erosions in particular] rather than the hyperketonaemia-related impairments in leukocyte function seem to be the primary factors predisposing the cow to *S. aureus* mastitis. The machine milking associated teat-end condition (callosity) may be an important constituent of the predisposition also in case of environmental pathogens (Neijenhuis et al., 2001). However, in the latter group a protective effect is attributed to the presence of monocytes and polymorphonuclear granulocytes in the milk of the teat cistern: also their functional capacity may play a significant role in the rapid elimination of invading pathogens, and so in diminishing the clinical consequences of the intramammary infection (Beaudeau et al., 2002). In the early days of lactation these components of the self-defence mechanisms must have been decreased by hyperketonaemia.

In summary, our results clearly demonstrate the predisposing role of hyperketonaemia to mastitis caused by environmental (mainly by GN) pathogens. However, as compared to the importance of teat-end lesions and other factors the hyperketonaemia-related predisposition to *S. aureus* mastitis seems to be negligible in group-fed high-yielding pp dairy cows.

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