Natural Antibodies Related to Energy Balance in Early Lactation Dairy Cows

A. T. M. van Knegsel,*†¹ G. de Vries Reilingh,* S. Meulenbergh,* H. van den Brand,*

J. Dijkstra,† B. Kemp,* and H. K. Parmentier*

*Adaptation Physiology Group, and †Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen University, PO Box 338, 6700 AH, Wageningen, the Netherlands

ABSTRACT

The objectives of this study were to determine the presence of natural antibodies (NAb) in plasma and milk of individual dairy cows and to study the relation between NAb concentrations and energy balance (EB) and dietary energy source. Cows ($n = 76$) were fed a mainly glucogenic, lipogenic, or a mixture of both diets (50:50 dry matter basis) from wk 3 before the expected calving date until wk 9 postpartum. Diets were isocaloric (net energy basis) and equal in intestinal digestible protein. Blood and milk were sampled weekly. Liver biopsies were taken in wk -2, 2, 4, and 6 relative to calving. Data are expressed as LSM $\pm$ SEM. The NAb titers are expressed as the $^2\log$ values of the highest dilution giving a positive reaction. The NAb concentration in plasma binding either keyhole limpet hemocyanin (KLH) or *Escherichia coli* lipopolysaccharide (LPS) increased with parity. The NAb concentration binding KLH was greater for cows fed the glucogenic diet (9.63 ± 0.08) compared with the lipogenic diet (9.26 ± 0.08). In milk, cows fed the glucogenic diet had smaller NAb concentrations binding KLH (3.98 ± 0.18) and LPS (2.88 ± 0.17) compared with cows fed the mixed diet (KLH: 4.93 ± 0.18 ; LPS: 3.70 ± 0.17). The NAb concentration in plasma had a positive relation with energy balance variables: EB, dry matter intake, milk yield, and plasma cholesterol, whereas NAb concentration in milk had a negative relation with energy balance variables: EB, dry matter intake, and plasma cholesterol. Additionally, NAb concentrations in milk had a positive relation with plasma nonesterified fatty acid concentration and milk fat and protein percentage. There was a tendency for a positive relation of NAb concentration binding LPS in plasma and somatic cell count in milk. No significant relations were detected between NAb concentrations in milk or plasma and plasma β -hydroxybu-

tyrate concentration and liver triacyl glyceride content. In conclusion, NAb are present in both milk and plasma of dairy cows peripartum and NAb concentrations increase with parity. Furthermore, our data indicate that a negative energy balance in dairy cows in early lactation can be associated with compromised innate immune function as indicated by decreased NAb concentration in plasma.

Key words: nutrition, energy balance, immune system, parity

INTRODUCTION

Lactation imposes serious metabolic demands for high-producing dairy cows in the periparturient period. A failure to meet the energy needs for milk production by energy intake results in a negative energy balance (NEB) that is in particular accompanied by metabolic disorders like fatty liver and ketosis (Grummer, 1993). Feeding extra glucogenic nutrients rather than lipogenic nutrients improved energy balance (EB; Van Knegsel et al., 2007a) and decreased plasma BHBA and liver triacylglyceride (TAG) concentrations, indicating a decreased risk for ketosis and fatty liver (Adler, 1970; Van Knegsel et al., 2005). Additionally, the early lactation period has been related with increased incidences of infectious diseases, like mastitis, endometritis, and laminitis (Heuer et al., 1999; Collard et al., 2000). These diseases have been related to suboptimal immune function in the periparturient period (Mallard et al., 1998), as illustrated by diminished mitogen-induced lymphocyte proliferation (Kehrli et al., 1989), reduced serum IgG and IgM concentrations (Detilleux et al., 1995; Lacetera et al., 2005), and diminished antibody responses (Mallard et al., 1997). Furthermore, several studies investigated the relation between NEB-related metabolic disorders and peripartum immune suppression in dairy cows. In vitro, it was shown that ketone bodies negatively affect the chemotactic and proliferative capacity of lymphocytes (Targowski and Klucinski, 1983; Nonnecke et al., 1992; Suriyasathaporn et al., 1999). Both ketone bodies (Franklin et al., 1991) and NEFA (Lacetera

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¹Corresponding author: Ariette.vanKnegsel@wur.nl

era et al., 2004) are shown to decrease mitogen-induced IgM secretion by leukocytes in vitro. In vivo, high BHBA concentration during a status of ketosis has been related to an increased severity of mastitis as indicated by bacterial counts (Kremer et al., 1993).

Natural antibodies (**NAb**) are defined as antigen-binding antibodies present in nonimmunized individuals and can be considered as a humoral part of the innate immune system. In mammals, NAb are preferentially derived from CD5⁺ B (B1) cells (Casali and Notkins, 1989) located in the peritoneal cavity (Ochsenbein et al., 1999), and along the intestinal tract (Quan et al., 1997). Natural antibodies are characterized by a broad specificity repertoire, with usually low binding affinity. In mammals, NAb are mostly of the IgM isotype class (Boes, 2000). In cooperation with the complement system, NAb might act as a first line of defense (Thornton et al., 1994) and can be regarded as the specific part of the innate immune system. Antigen uptake, processing, and presentation via B cells or dendrites may be enhanced by NAb, which provide initial protection against infection. Natural antibody concentrations increased during aging in dairy cows (Srinivasan et al., 1999) and chickens (Parmentier et al., 2004). This indicates that NAb are the cumulative result of antigenic stimulation of the poly-specific receptors of B1 B cells (Tomer and Shoenfeld, 1988).

Little is known about the existence and function of NAb in dairy cows in the peripartum period. The objectives of the present study were firstly to determine the presence of NAb binding keyhole limpet hemocyanin (**KLH**) and *Escherichia coli* LPS in plasma and milk of high-producing dairy cows in the transition period. The second objective was to study the relation between NAb concentration and EB and indicators of metabolic disorders, like BHBA and liver TAG concentration. The third objective was to study the effect of dietary energy source on NAb concentration. The overall experiment had been designed to study the effect of dietary energy source during the transition period and early lactation on EB, metabolites, metabolic hormones, and days till first ovulation in high-producing dairy cows. These results were described earlier (Van Kneegsel et al., 2007b).

MATERIALS AND METHODS

Experimental Design

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol. The experimental design, ingredient, and chemical composition of the diets, calculation of EB, and analytical procedures for determination of plasma NEFA, BHBA, cholesterol, glucose, and insulin concentrations, and liver TAG content were previously re-

ported (Van Kneegsel et al., 2007b). Cows (n = 76) were blocked by parity, calving date, and milk production in the previous lactation (multiparous cows) or expected milk production based on pedigree (primiparous cows) and assigned to a mainly lipogenic (n = 26), a mainly glucogenic (n = 25) diet, or mix of both diets (50:50 DM basis; n = 25) from wk 3 before the expected calving date until wk 9 postpartum (**pp**). Basic diet did not differ among treatments and consisted of grass silage, corn silage, chopped alfalfa hay, and rapeseed meal (48:45:3:3 DM basis). Corn and milocorn or rumen-protected fat and citrus pulp were the main concentrate ingredients of the glucogenic and lipogenic concentrates, respectively. Concentrate and forage was supplied separately (38:62 DM basis). Diets were isocaloric (net energy basis; VEM system; Van Es, 1975) and equal in intestinal digestible protein and degradable protein balance (DVE/OEB system; Tamminga et al., 1994). Cows were housed in a free-stall with slatted floor and cubicles and milked twice daily (0600 and 1700 h).

All cows were monitored for milk production, BW, and plasma metabolites and metabolic hormones. A random subset of cows was monitored for feed intake (n = 72) and liver TAG content (n = 42). Blood samples were obtained weekly from wk -3 (minimal 3 d after feeding first experimental diet) until wk 9 pp, 2 to 3 h after the a.m. feeding. Blood samples were stored on ice for maximal 2 h. Plasma was obtained by centrifugation (10 min at 2,900 × g), aliquoted, and frozen at -20°C until analysis. From wk 2 until wk 9 pp, at the same day as the blood samples, milk was sampled during the p.m. milking and stored at -20°C until analysis of NAb. Liver biopsies were obtained in wk -2, 2, 4, and 6 relative to week of calving.

Analytical Procedures

Total immunoglobulin titers (NAb titers) binding KLH (Cal Biochem-Novabiochem Co., San Diego, CA) and *Escherichia coli* O55:B5 LPS (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were determined by indirect ELISA in plasma and milk of all cows. Ninety-six-well plates were coated with 1 µg of KLH/mL or 4 µg of LPS/mL (100 µL/well) dissolved in carbonate buffer (pH 9.6). The plates were incubated overnight at 4°C and then washed twice with water and 0.05% Tween. Plates for NAb determination in milk were blocked with 2.5% rabbit serum in PBS and 0.05% Tween. Serial dilutions of plasma (1:4) or milk (1:3) in PBS, 0.05% Tween, and 2.5% rabbit serum were added, dilutions started at 1/40 for plasma and 1/30 for milk samples. Plates were incubated for 1 h at room temperature. After being washed with water and 0.05% Tween,

Table 1. Distribution of cows (n = 76) per diet per parity class

Parity	Diet			Total
	Glucogenic	Mixed	Lipogenic	
1	7	7	7	21
2	8	7	7	22
3	5	5	7	17
≥4	5	6	5	16
Total	25	25	26	76

binding of antibodies was detected using 1:10,000 diluted rabbit-anti-bovine IgG (whole molecule) conjugated to peroxidase (RAB/IgG_{H+L}/PO) (Sigma-Aldrich Chemie GmbH). After being washed, tetramethylbenzidine (Sigma-Aldrich Chemie GmbH) as a substrate, and 0.05% H₂O₂ were added and incubated for 10 min at room temperature. The reaction was stopped by adding 1.25 M H₂SO₄. Extinctions were measured with a Multiskan reader (Labsystems, Helsinki, Finland) at a wavelength of 450 nm. Titers were expressed as the ²log values of the highest dilution giving a positive reaction.

Statistical Analysis

Repeated measures ANOVA [PROC MIXED (Littell et al., 1996) of SAS Version 9.1; SAS Institute Inc., Cary, NC] was performed for EB, DMI, milk yield and composition, metabolites and metabolic hormones, concentration of NAb binding KLH, and concentration of NAb binding LPS measured in milk or plasma. Diet (glucogenic, lipogenic, or mixed), week (−3 to 9 pp), parity (1, 2, 3, and ≥4), and a diet × parity interaction (D × P) were included in the model as fixed effects (model 1). Parity was distributed over 4 classes to obtain a balanced distribution of cows per class (Table 1). Preliminary analysis showed no significant effect of diet × week interaction and was therefore not included in model 1. A first-order autoregressive structure was the best fit and was used to account for within-cow variation. Model assumptions were evaluated by examining the distribution of residuals. Values are presented as LSM with their SEM. Second, to test relations between NAb concentration (binding KLH or LPS) and EB, DMI, milk production, and metabolites, these variables were included as fixed effects in a repeated measurements model (model 2) to obtain regression coefficients (β). To avoid collinearity, week, diet, and parity were not included in model 2.

RESULTS

Energy Balance and Metabolites

Earlier we presented the effects of glucogenic vs. lipogenic and mixed diet on EB, DMI, milk yield and compo-

sition, metabolites, and metabolic hormones for the prepartum and postpartum period and multiparous and primiparous cows separately (Van Knegsel et al., 2007b). Table 2 presents a summary of these results. Whereas DMI, milk yield, and milk protein content did not differ among diets, cows fed the glucogenic diet had a smaller milk fat percentage; less negative EB; smaller plasma NEFA, BHBA, and cholesterol concentration; and postpartum a greater plasma insulin concentration ($P < 0.01$). Somatic cell count in milk was greater for cows fed the lipogenic diet ($P < 0.05$) compared with the other diet groups and decreased ($P < 0.01$) with week pp, in particular for cows fed the lipogenic diet.

NAb Related to Diet and Parity

The NAb activity was present in both plasma and milk for all cows. In plasma, NAb concentration binding KLH was smaller ($P < 0.05$) for cows fed the lipogenic diet compared with cows fed the glucogenic diet (Table 3). In plasma, NAb concentrations binding to KLH and LPS decreased prepartum and increased from week 2 pp (Figure 1). In milk, NAb concentrations binding KLH and LPS were greater for cows fed the mixed diet compared with the other diet groups ($P < 0.05$) and decreased from wk 2 until wk 9 pp (Figure 2). In plasma, NAb concentrations binding KLH increased with parity. The NAb concentration binding LPS increased with parity for cows fed lipogenic and glucogenic diet, but decreased with parity for cows fed the mixed diet (Figure 3).

NAb Related to EB and EB-Related Metabolites

In plasma, NAb concentration binding KLH had a positive relation ($P < 0.05$) with EB, DMI, milk yield, milk lactose, and plasma cholesterol and a negative relation with milk protein percentage (Table 4). The NAb concentration binding LPS tended ($P < 0.10$) to have a positive relation with EB, SCC, and plasma cholesterol. Relations of EB and EB-related variables of NAb concentrations in milk were opposite to relations found in plasma, except for plasma glucose and insulin. In milk, NAb concentrations binding KLH had a negative relation ($P < 0.05$) with DMI, EB, plasma cholesterol, glucose, and insulin and a positive relation with milk fat and protein percentage and plasma NEFA. In milk, NAb concentrations binding LPS had a negative relation with EB, DMI, and plasma cholesterol and a positive relation with milk protein percentage and plasma NEFA.

Table 2. Energy balance, DMI, milk production and composition, metabolites and metabolic hormones for dairy cows fed a glucogenic (n = 25), a lipogenic (n = 26), or mixed diet (n = 25; LSM $\pm$ SEM)¹

Item	Diet			SEM	P-value			
	Glucogenic	Mixed	Lipogenic		Diet	Week	Parity	Diet $\times$ parity
Energy balance (kJ/kg ^{0.75} ·d)	-38 ^a	-103 ^b	-76 ^{ab}	15	0.01	<0.01	<0.01	0.03
DMI (kg/d) ¹	20.2	20.0	20.5	0.3	0.40	<0.01	<0.01	0.92
Milk yield (kg/d)	40.2	40.1	39.7	0.8	0.88	<0.01	<0.01	0.63
Milk fat (%)	3.86 ^a	4.10 ^{ab}	4.16 ^b	0.07	<0.01	<0.01	<0.01	0.65
Milk protein (%)	3.26	3.26	3.26	0.04	0.99	<0.01	0.56	0.78
Milk lactose (%)	4.62 ^a	4.60 ^a	4.53 ^b	0.02	<0.01	<0.01	<0.01	0.28
SCC, ² $\times 10^3$	3.60 ^a	3.67 ^a	4.25 ^b	0.16	<0.01	<0.01	<0.01	<0.01
NEFA (mMol/L)	0.23 ^a	0.29 ^b	0.29 ^b	0.01	<0.01	<0.01	<0.01	<0.01
BHBA (mMol/L)	0.63 ^a	0.73 ^b	0.76 ^b	0.03	<0.01	<0.01	0.40	0.02
Cholesterol (mMol/L)	3.55 ^a	4.44 ^b	4.73 ^b	0.16	<0.01	<0.01	0.16	0.12
Glucose (mMol/L)	3.43	3.45	3.40	0.03	0.44	<0.01	<0.01	0.73
Insulin (uIU/mL)	4.39	4.04	3.64	0.28	0.16	<0.01	0.11	0.18
Liver TAG ³ (mg/g of wet weight)	25.8	49.0	49.8	7.6	0.06	<0.01	0.35	0.03

^{a,b}Values in the same row with different superscripts differ ($P < 0.05$).

¹Energy balance and milk variables represent LSM for wk 1 to wk 9 relative to calving. Metabolic variables represent LSM for wk -3 to wk 9 relative to calving. Energy balance and DMI data are based on 23 vs. 25 vs. 24 cows.

²Natural logarithm $\times 10^3$ /mL.

³Liver triacylglycerides (data are based on 12 vs. 17 vs. 13 cows).

Relation Between NAb Concentrations in Plasma and Milk

There was a tendency for a negative relation between NAb in plasma and NAb in milk for both NAb binding KLH ($\beta = -0.101$; $P = 0.07$) and NAb binding LPS ($\beta = -0.100$; $P = 0.12$).

DISCUSSION

In the present study we detected binding of antibodies to the model antigens KLH (glycoprotein) and LPS in plasma and milk of dairy cows in the peripartum period that had not been intentionally immunized with these components. In general, antibodies from nonimmunized individuals, when detected in specific binding assays such as ELISA, are regarded as background (Ochsenbein et al., 1999). Conversely, it is known that next to antigen-specific antibodies, nonspecific antibodies, with no or unknown binding specificity (NAb) are

present in the blood of healthy people and laboratory rodents (Dacie, 1950), fish (Gonzalez et al., 1988), and poultry (Parmentier et al., 2004). The NAb are probably derived from CD5+ B(1) cells (Casali and Notkins, 1989). Because approximately 20% of the peripheral B cells are CD5+, NAb form an important part of the antibody repertoire as well as the mass of immunoglobulins in mammals. As earlier discussed for poultry (Star et al., 2007), the major difference between responses of NAb binding KLH or NAb binding LPS is that cows did not and probably will not encounter KLH, thus reflecting a capacity to respond, whereas cows probably did encounter LPS; thus, the latter reflects an active status of the innate immune system.

The NAb activity was detected in all cows, and NAb concentrations in plasma increased with parity. This indicates a relation between parity and the capacity of cows to produce NAb in plasma and milk. Indeed, Srinivasan et al. (1999) found greater concentrations

Table 3. Natural antibody in plasma and milk binding keyhole limpet hemocyanin (KLH) and LPS in milk in the transition period for dairy cows fed a glucogenic, a lipogenic, or a mixed diet (LSM $\pm$ SEM)

Item	Diet			SEM	P-value			
	Glucogenic	Mixed	Lipogenic		Diet	Week	Parity	Diet $\times$ parity
In plasma, wk -3 until wk 9								
KLH	9.63 ^a	9.40 ^{ab}	9.26 ^b	0.08	<0.01	<0.01	<0.01	0.11
LPS	8.53	8.56	8.40	0.07	0.22	<0.01	0.13	0.01
In milk, wk 2 until wk 9								
KLH	3.98 ^a	4.93 ^b	4.39 ^a	0.18	<0.01	<0.01	0.81	0.80
LPS	2.88 ^a	3.70 ^b	3.33 ^a	0.17	<0.01	<0.01	0.44	0.19

^{a,b}Values in the same row with different superscripts differ ($P < 0.05$).

Figure 1. The natural antibody (NAb) concentration binding key-hole limpet hemocyanin (KLH; A) or LPS (B) in plasma for all cows from wk -3 to wk 9 relative to calving. Cows were fed a glucogenic, a lipogenic, or a mixed (50:50 DM basis) diet. Values represent means per diet per week.

Figure 2. The natural antibody (NAb) concentration binding key-hole limpet hemocyanin (KLH; A) or LPS (B) in milk for cows from wk 2 to wk 9 relative to calving. Cows were fed a glucogenic, a lipogenic, or a mixed (50:50 DM basis) diet. Values represent means per diet per week.

of NAb binding mannans in adult cows compared with calves and newborn calves. The origin of NAb is a subject of debate. The NAb repertoire and concentrations may be shaped by continuous polyclonal stimulation by exogenous microbes initiating cross reactivity driven responses of auto-reactive B cells or may correspond with the secretion of naturally occurring (auto-)reactive B cell clones, or both (Avrameas, 1991). The positive effect of parity on NAb concentration in cows corresponds with the idea that exogenous stimuli enhance the NAb formation (Prokesova et al., 1996).

The function of NAb in bovine milk, produced during wk 2 until wk 9 relative to calving, as reported in the current study, remains unspecified. It can be hypothesized that if calves are able to absorb IgG, or NAb in particular, after the intestinal closure at about 24 h of age (Rajala and Castren, 1995), NAb in milk might contribute to the immune competence of young calves. Although concentration of IgG in milk rapidly declines during the first 3 d pp (Stott et al., 1981), a possible

immunological value for NAb in milk in later stages of lactation is confirmed by the expression of the FcRn receptor, neonatal IgG transporter, in the small intestine of adult cows (Kacskovics et al., 2000). This indicates that also after the intestinal closure soon after birth, the ruminant is still able to selectively transport IgG across the intestinal wall via the FcRn receptor. This is in accordance with the human FcRn receptor, which has been detected in both fetal and adult intestinal epithelial cells (Israel et al., 1997), whereas in rodents expression of the FcRn in intestinal cells is limited to the suckling period (Martin et al., 1997). Alternatively, it can be speculated that NAb in milk are a residue of the immune barrier in the mammary gland and therefore contribute to the resistance to mammary infections.

The relation between nutrition and immune function in dairy cows is complicated and remains a subject of interest (Goff, 2006). Recently, beneficial effects have been reported of glutamine supplementation (Doepel et

Figure 3. The natural antibody (NAb) concentration binding keyhole limpet hemocyanin (KLH; A) and LPS (B) in plasma for cows from wk -3 to wk 9 relative to calving per diet per parity class. Cows were fed a glucogenic, a lipogenic, or a mixed (50:50) diet. Values represent weekly observations averaged per diet per parity class.

al., 2006) and polyunsaturated fatty acids (Lessard et al., 2003, 2004) on the immune response in dairy cows in early lactation. Also parity $\times$ supplement interactions were observed. These effects, however, were limited and were sometimes ambiguous. Further, grain-induced subacute ruminal acidosis (SARA) has been related to increased lysis of gram-negative bacteria and increased concentration of LPS in the rumen of lactating dairy cows (Gozho et al., 2007). Earlier, increased ruminal LPS concentration has been related to an increase in peripheral plasma LPS concentration in calves (Aiumlamai et al., 1992). In the current study, we aimed for increasing the glucogenic nutrient content in the diet by feeding rumen resistant starch, trying to avoid cows suffering from SARA. In an earlier experiment (Van Knegsel et al., 2007a), we reported with similar diets no diminishing effect of the glucogenic diet on the secretion of short chain fatty acids in milk, indicating no decrease in de novo fatty acid synthesis as established by lowering of ruminal pH associated with SARA. This might imply that, although we fed a glucogenic diet, because this diet was especially high in rumen resistant starch, cows had not an increased risk for SARA and therefore did not experience an increase in ruminal LPS concentration. With high concentrations of dietary rumen resistant starch, starch fermentation is high in the intestine and cows are possibly more at risk for an increase in LPS at the intestine level. Nevertheless, there were no indications for high LPS concentration in the intestine, and no diet-related effect on NAb binding LPS could be detected. We found greater NAb concentrations binding KLH for cows fed the glucogenic diet compared with cows fed the lipogenic diet. As unex-

Table 4. Regression coefficients (β) of natural antibody concentrations binding keyhole limpet hemocyanin (KLH) and LPS, related to energy balance, DMI, milk production and composition, metabolites, and metabolic hormones in dairy cows from wk -3 to wk 9 relative to calving

Item	Plasma		Milk	
	KLH	LPS	KLH	LPS
Energy balance (kJ/kg ^{0.75} .d)	0.001**	0.0004*	-0.002**	-0.002**
DMI (kg/d)	0.039**	0.013	-0.059**	-0.054**
Milk yield (kg/d)	0.015**	0.006	-0.016	-0.013
Milk fat (%)	-0.032	-0.058	0.207**	0.196*
Milk protein (%)	-0.287**	-0.210*	1.197**	1.297**
Milk lactose (%)	0.536**	0.087	0.006	-0.092
SCC, ¹ $\times 10^3$	-0.015	0.042*	0.020	0.046
NEFA (mMol/L)	-0.252	-0.028	0.686**	1.079**
BHBA (mMol/L)	-0.120	-0.067	0.179*	0.001
Cholesterol (mMol/L)	0.102**	0.038*	-0.205**	-0.196**
Glucose (mMol/L)	-0.051	-0.023	-0.212**	-0.116
Insulin (uIU/mL)	-0.003	0.003	-0.070**	-0.008
Liver TAG ² (mg/g of wet weight)	-0.002	-0.001	0.005	0.001

¹Natural logarithm $\times 10^3$ /mL.

²TAG = triacylglycerides.

** $P < 0.05$; * $P < 0.10$.

Figure 4. Calculated energy balance (in kJ/kg^{0.75}·d) for cows from wk 1 to wk 9 relative to calving per diet per parity class. Cows were fed a glucogenic, a lipogenic, or a mixed (50:50) diet. Values represent LSM $\pm$ SEM.

pected, this diet effect was already present in the first plasma sample that was taken 3 d after feeding the first experimental diet. Information on the NAb concentration before feeding is lacking, but rapid effects at 5 d after start of dietary supplementation (polyunsaturated fatty acids) on immune variables were reported earlier (Lessard et al., 2003). Cows fed the glucogenic diet had not only greater NAb concentrations binding KLH, but also a significant improved EB compared with the other diet groups, which supports the hypothesis that in early lactation the severity of the NEB can be related to innate immune function.

The NAb concentrations binding LPS increased with parity for cows fed the lipogenic and glucogenic diet, in contrast to cows fed the mixed diet that had smaller NAb concentrations for the greater parity classes (Figure 3). Furthermore, third and greater parity cows fed the mixed diet had an extreme NEB, compared with the other diet groups (Figure 4). Confirmed by the presented relation between NAb concentration binding LPS and EB, the decrease with parity in NAb concentration binding LPS for the mixed diet might be related to the extreme negative energy status as calculated for these diet $\times$ parity groups. In accordance with this indication, Figure 3a shows that also NAb binding KLH hardly increased ($P > 0.05$) with parity for cows fed the mixed diet, in contrast to the other diets, although a diet $\times$ parity interaction was not detected for NAb binding KLH.

Earlier, several studies associated EB or EB-related variables in early lactation dairy cows with indicators of humoral (Mallard et al., 1997) or cellular (Kehrli et al., 1989; Lacetera et al., 2005) adaptive immune function. Not only a negative energy status, but also NEB-related metabolic disorders like ketosis (Franklin et al., 1991; Suriyasathaporn et al., 1999) and fatty

liver (Bobe et al., 2004), have been suggested to be related to impaired immune function, as indicated by decreased antibody responses (Wentink et al., 1997), impaired neutrophil function (Hoeben et al., 2000), decreased capacity for phagocytosis by macrophages (Zerbe et al., 2000), and a depression of interferon production by leukocytes (Szuster-Ciesielska et al., 1995). The objective of the current study was to relate EB and plasma metabolites to innate immune function in vivo as indicated by NAb concentrations to find evidence for NEB-associated innate immune suppression in early lactation dairy cows. Indeed, we found relations between NAb concentrations and EB, DMI, and milk yield. In addition, plasma NEFA, which is considered as an indicator for body fat mobilization and NEB, was related to NAb concentrations in milk. Although, decreased plasma insulin concentration stimulates lipolysis and NEB, insulin was not related to NAb concentrations. Furthermore, in contrast to earlier studies that related immune suppression to metabolic disorders (e.g., Wentink et al., 1997), no relations were detected between NAb concentrations and indicators for NEB-associated metabolic disorders ketosis (BHBA) and fatty liver (liver TAG content). This might imply that a cow in a status of NEB might not be able to maintain its concentrations of humoral natural immunity, though the innate immunity seems not to be affected by postpartum metabolic disorders, like ketosis and fatty liver. On the other hand, the important role of NAb in the direction of TH-1 or TH-2 mediated specific immunity (Bayry et al., 2004, 2005), activation of B cells, and adjuvating effect on cellular responses (Stager et al., 2003) may be related with the increase in infectious diseases in early lactation. In this respect, it is noteworthy that cows fed a glucogenic diet were characterized by greater NAb concentrations binding KLH in plasma and smaller SCC, whereas cows fed a lipogenic diet were characterized by smaller NAb concentrations binding KLH and a greater SCC.

Indicators for immune competence or health status are of importance in dairy practice. Somatic cell count has been considered as an indicator for udder health, and therefore the trend for a positive relation between NAb concentration binding LPS and SCC is interesting, but should be confirmed in further studies. There were 9 incidences of clinical mastitis detected during the first 9 wk of lactation. Clinical mastitis was related to a numerically, but not significantly, increased concentration of NAb binding LPS compared with nonmastitis cows in both plasma [8.48 ± 0.02 vs. 8.57 ± 0.32 (mean $\pm$ SE); $P = 0.84$] and milk [2.98 ± 0.06 vs. 3.40 ± 0.54 (mean $\pm$ SE); $P = 0.14$]. If NAb concentration would turn out to be a good indicator for immune function in early lactation dairy cows, it can still be questioned

how to interpret the negative relation between NAb concentration in plasma and NAb concentration in milk. Furthermore, whereas NAb in plasma had a positive relation with several energy balance indicators (EB, DMI, milk yield, and plasma cholesterol), concentration of NAb in milk turned out to have a negative relation with the majority of determined energy balance variables (EB, DMI, and plasma cholesterol). This is in accordance with tendencies for an inverse relation between NAb concentration in plasma and NAb concentration in milk. Based on these observations, it can be hypothesized that the partitioning of NAb between plasma (cow) and milk (calf) parallels the partitioning of energy between body reserves and milk as observed in early lactation dairy cows. It has been suggested that the current high-producing dairy cow has a tremendous priority in early lactation for her calf at the cost of cow's body condition, health, and fertility (Friggens, 2003), as indicated by high milk yield, that results in a NEB and extensive body fat mobilization. The current data indicate that this extensive priority for her current calf seems not only to be reflected in milk yield, but also in high NAb concentrations in milk in the first weeks of lactation while simultaneously NAb concentrations in plasma are decreased. The physiological mechanism that implements this suggested priority for milk yield is considered to be a status of hypoinsulinemia in early lactation (Bonczek et al., 1988) that reduces glucose uptake by muscle and adipose tissue and makes glucose available for the mammary gland, which is not insulin-responsive (Bauman and Elliot, 1983). The physiological mechanism that implements this hypothesized priority for high NAb concentrations in milk is unknown, although the reverse relation between NAb in plasma and milk indicates an active mechanism in regulating the concentration of NAb in milk. A possible candidate might be the FcRn receptor, as transcripts of this receptor were identified in the bovine mammary gland and suggested to play a role in regulating IgG transfer into milk (Kacskovics et al., 2000). In spite of this, theories concerning physiological mechanisms, which establish the partitioning of NAb to milk are as yet speculative.

CONCLUSIONS

From the present data, we conclude that NAb are present in dairy cows in early lactation. Apart from the increase with parity, indicating environmental sensitization, NAb concentrations binding KLH and LPS in plasma had a positive relation with EB. Hereby, our data indicate that a NEB in dairy cows in early lactation can be associated with compromised innate immune function. The possible role of NAb in regulation of the immune response raises the question whether effects

on the concentration of NAb underlie immune-related health problems in dairy cows during early lactation. This implies that feeding diets to dairy cows to maintain NAb concentrations may favor maintenance of health. Further studies are in progress to unravel the relations among nutrition, NAb, SCC, and various biochemical plasma variables.

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