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Effects of different nutrient supply on metabolism and mammary immune response to an LPS challenge in early lactation of dairy cows

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ABSTRACT

Energy and nutrient deficiency in dairy cows in early lactation is considered to contribute to their increased susceptibility to mastitis. We have tested the hypothesis that feeding diets with high contents of either nitrogenic, glucogenic, or lipogenic components in early lactation affects both the endocrine and metabolic status, as well as the mammary immune competence. After calving, cows were fed increasing amounts of concentrate up to 10 kg/d rich in crude protein (nitrogenic, n = 10), glucogenic precursors (glucogenic, n = 11), or lipids (lipogenic, n = 11). In wk 3, one udder quarter was challenged with lipopolysaccharide (LPS) from *Escherichia coli*. Blood and milk were sampled on the day before LPS challenge (d −1), and on d 0, 1, 2, 3, and 9 after LPS challenge. On the day of LPS challenge additional samples were taken hourly for quarter milk and every 3 h for blood. Urea concentrations were higher in plasma and milk of cows fed the nitrogenic diet. However, plasma concentrations of glucose, cholesterol, triglycerides, β-hydroxybutyrate, nonesterified fatty acids, as well as insulin, glucagon, and insulin-like growth factor-1 were not affected by the different diets. The mammary immune challenge induced a substantial increase of somatic cell count (SCC) in the treated quarter, and a transient decrease of total milk yield and white blood cells similar in all diet groups for one day. The absolute phagocytosis of blood leukocytes was decreased; however, the phagocytosis per cell was increased in glucogenic-fed cows at 6 h after LPS challenge. During mammary inflammation an insulin resistance, shown by increased plasma glucose, insulin, and glucagon, developed similarly in all diet groups. β-hydroxybutyrate and nonesterified fatty acids were decreased at 1 d after LPS challenge in glucogenic-fed cows only. Cholesterol did not change, and triglycerides only decreased significantly in lipogenic-fed cows 6 h after challenge. On d 9 after LPS challenge, SCC and milk yield and metabolic factors were recovered in all groups. In conclusion, the endocrine and metabolic situation, and the immune response to intramammary LPS of dairy cows during early lactation was not substantially influenced by the elevated supply of nitrogenic, glucogenic, or lipogenic components due to the provided feed in this study.

Key words: dairy cow, early lactation, diet composition, mastitis, metabolism

INTRODUCTION

In dairy cows, the susceptibility to IMI is particularly high immediately after parturition (Burton and Erskine, 2003; Ingvartsen and Moyes, 2015). An influence of the nutritional and metabolic status on the susceptibility to mastitis during this period is most likely, as it is the most challenging time for the metabolism of the dairy cow, even when kept under optimal management conditions. With the beginning of lactation, the dairy cow undergoes remarkable endocrine and metabolic changes that are required to meet the nutrient and energy demand for a high level of milk production. Together with a reduced feed intake around calving this leads to a negative energy balance (**NEB**). Simultaneously, cows are immunosuppressed (Kehrli et al., 1989; Horst et al., 2021). The NEB was identified as one of the reasons for the immunosuppression and elevated risk of periparturient diseases (Drackley et al., 2007; van Knegsel et al., 2014; Ingvartsen and Moyes, 2015). To counteract the NEB, cows mobilize body reserves, mostly body fat and, to a lesser extent, body protein. The metabolic effects of an NEB are low plasma glucose and insulin concentrations and high concentrations of plasma nonesterified fatty acids (**NEFA**), BHB, acetone, acetoacetate, and liver triglycerides (van Knegsel et al., 2007; Gross et al., 2011a,b). Elevated BHB concentrations have been shown to suppress the activity of immune cells of periparturient cows (Hoeben et al., 1997), and in vitro BHB was shown to reduce leukocyte chemotaxis (Suriyasathaporn et al., 1999). Furthermore, high BHB levels in plasma have been positively related to

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the severity of mastitis as indicated by bacterial counts (Kremer et al., 1993) and infused BHB reduced the increase of SCC during an intramammary LPS challenge (Zarrin et al., 2014a,b). In addition, cows with high serum NEFA concentrations prepartum or postpartum were also shown to have an increased incidence of intramammary infections (Dyk et al., 1995; Holtenius et al., 2004).

The nutrient availability and hence metabolic effects can be manipulated by ingredients in the diet (van Knegsel et al., 2005, 2007). Feeding nitrogenic diets implies the supply of nitrogen and protein-rich components such as rapeseed and soybean meal, urea, peas, and young herbage. Glucogenic nutrients, such as grain, nonfiber carbohydrates, or propylene glycol, are mainly fermented in the rumen, result in the production of propionate and increase plasma glucose and insulin levels (van Knegsel et al., 2005, 2007). Furthermore, they induce decreased NEFA, BHB, and triglyceride levels (Studer et al., 1993; Minor et al., 1998; Drackley et al., 2005). Feeding lipogenic nutrients was shown to increase plasma NEFA and BHB concentrations, and led to decreased plasma glucose and insulin concentrations (van Knegsel et al., 2005).

Therefore, the aim of the present study was to investigate if manipulation of the nutrient supply by dietary alterations (i.e., feeding a nitrogenic, glucogenic, or lipogenic diet) can affect the nutritional and metabolic status and improve the immune response in dairy cows in early lactation. We hypothesized that cows fed a glucogenic diet are more successful in coping with an LPS-induced mastitis compared with cows fed a nitrogenic or lipogenic diet. Results could help to improve udder health in early lactation of dairy cows by different feeding strategies.

MATERIALS AND METHODS

Animals, Housing, and Feeding

Animal trials followed the Swiss Law on Animal Protection and were approved by the Committee of Animal Experiments of the Canton Fribourg, Switzerland (approval no. 2018–35-FR). Thirty-two dairy cows [Holstein (n = 20), Red Holstein (n = 9), and Red Holstein × Simmental (n = 3)] in their second to eighth lactation [parity 3.7 ± 1.7, (mean ± SD)] housed at the Agroscope research station (Posieux, Switzerland) were enrolled. Except for the time around calving when they were kept in individual calving pens with straw bedding, cows were housed in the tiestall barn throughout the experiment. Machine milking was performed twice daily at 0530 and 1600 h. Cows were offered hay and water ad libitum and minerals (50 g/cow) were supplied daily. Concentrate was given thrice daily according to the experimental design.

Experimental Design

Cows were distributed into 3 groups with similar mean performance and BW based on milk yield (mean ± SD; 9,231 ± 1,519 kg of milk) and BW (688 ± 52 kg) in their previous lactation. Each group was assigned to a specific diet: nitrogenic [emphasis on CP supply (n = 10)], glucogenic [emphasis on carbohydrates supply (n = 11)], or lipogenic [emphasis on lipids supply (n = 11)]. For detailed composition of concentrates see Tables 1 and 2. From 3 weeks before expected calving until calving cows were fed a mixed diet consisting of a concentrate combining the components of all 3 specific concentrates mentioned above (mixed, 1.5 kg/d) and a compensation concentrate for dry cows (**AG**, 1.5 kg/d). All diets were formulated to cover the energy requirements for maintenance and pregnancy (mixed diet) or milk production (specific diets). After calving, diet was switched to the assigned specific diet meaning cows were fed the specific concentrate (3.5 kg/d) and a compensation concentrate for lactation (**AL**, 1.5 kg/d). Total concentrate was raised to a total of 7.5 kg/d (6 kg of specific concentrate + 1.5 kg of AL) at the beginning of wk 2 of lactation (7 ± 2 DIM) and to a total of 10 kg/d (8.5 kg of specific concentrate + 1.5 kg of AL) at the beginning of wk 3 of lactation (14 ± 2 DIM). In the third week of lactation (18 ± 3 DIM, referred to as d 0) when the cows were still supposed to be in a NEB all cows were challenged with 20 μg of LPS intramammarily into the right front quarter (n = 24) or the right rear quarter (n = 8) after morning milking. The quarter was selected based on the SCC on the treatment day to ensure similar low SCC below 120 cells/mL in all challenged quarters. The LPS (from *Escherichia coli* serotype O26:B6, Sigma Aldrich) was diluted in 10 mL of sterile saline solution (9 g/L) and administered through the teat canal into one quarter. The contralateral healthy quarter was used as control and received 10 mL of saline solution.

Blood and Milk Sampling. On the day before LPS challenge (d −1) milk samples were taken at −25 and −15 h, and blood samples at −18 h. On the day of LPS challenge (d 0), sampling was performed immediately before challenge (−1 h) and hourly until 9 h for quarter milk samples, and every 3 h for blood samples. Total milk samples were collected at milking (−1 and 9 h after challenge). Further sampling was performed on the day after challenge at 23 h and on d 2, 3, and 9 after challenge.

Blood samples were always taken in the morning after milking and before feeding. They were collected via

Table 1. Composition of concentrates used to mix rations fed during the experiments before and after calving in the various treatment groups within

Concentrate[1]	Composition
AG	46% soybean meal, 26% rapeseed meal, 23.6% barley, 2.7% NaCl, 1.3% magnesium oxide, 0.4% premixture for dry cows (vitamins, bulk and trace elements)
AL	45% soybean meal, 25% rapeseed meal, 20.6% barley, 5% calcium carbonate, 2.7% NaCl, 1.3% magnesium oxide, 0.4% premixture for lactating cows (vitamins, bulk and trace elements)
Mixed	25% soybean meal, 23.3% corn, 13.3% barley, 11.7% rapeseed meal, 11.7% beet pulp, 6.7% wheat, 5% rumen-protected fat, 3.3% rapeseed press cake
Glucogenic	40% corn, 20% wheat, 10% barley, 10% soybean meal, 10% rapeseed meal, 10% beet pulp
Nitrogenic	55% soybean meal, 15% rapeseed meal, 10% barley, 10% corn, 10% beet pulp
Lipogenic	20% barley, 20% corn, 15% beet pulp, 15% rumen-protected fat, 10% soybean meal, 10% rapeseed meal, 10% rapeseed press cake

[1]AG = compensation concentrate for dry cows; AL = compensation concentrate for lactation.

jugular vein puncture using evacuated tubes coated with either K3EDTA, Clot Activator (both VACUETTE, Greiner Bio-One International GmbH) or Li-Heparin (Vacutainer, BD) depending on further purpose of the sample and were cooled on wet ice until further use. Blood plasma or serum was received by centrifugation (3,000 × g, 20 min, 4°C) and stored at −80°C until analysis.

Proportional whole udder milk samples for gross composition analysis were collected proportionally to milk yield twice daily on sampling days during machine milking. These milk samples were stabilized with bronopol and sent to a commercial laboratory (Suisselab, Zollikofen, Switzerland) for analysis of fat, protein, lactose, urea, and SCC. On the day of LPS challenge, additional quarter strip milk samples (10 mL) of both the LPS and control quarters were taken by hand. Aliquots of 5 mL and 1 mL of all milk samples were stored at −80°C for further analysis.

Rectal Temperature. Rectal temperature was measured simultaneously to the quarter milk sampling on the day of LPS challenge.

Milk Yield, Feed Intake, BW, Energy Balance. After calving, milk yields of individual milking as well as feed intake was recorded daily. Body weight was measured at least once weekly directly after milking. Dry matter content of feed samples was measured and feed samples were analyzed for chemical composition as part of routine analyses performed by the Agroscope research station. For details on chemical analyses of feed samples see Table 2. Dry matter intake was calculated by multiplying the fresh matter intakes of hay and concentrates with the respective DM content. By multiplying the percentage of milk constituents with milk yield the total mass of milk constituents was calculated. Energy balance was calculated based on the equations provided by Agroscope (2016) considering the energy intake and the milk production.

Laboratory Procedures

The white blood cell (**WBC**) count and differential blood count of leukocytes were determined from whole blood anticoagulated with K3EDTA using a hematology analyzer (Vetscan HM5, Abaxis Inc.).

Leukocyte phagocytosis in blood was assayed using PHAGOTEST-Kit (Celonic Deutschland GmbH) according to the manufacturer's instructions using an imaging reader (Cytation 5 Cell imaging Multi-Mode Reader, BioTek Instruments Inc.) for quantification of phagocytosis by fluorescence (485 nm; 528 nm). Absolute phagocytosis (difference in fluorescence between test and control samples) and phagocytosis per neutrophil granulocyte (absolute phagocytosis divided by number of neutrophil granulocytes) was calculated using the results from the imaging reader and the hematology analyzer.

Glucagon in plasma was measured by a commercially available RIA (cat. no. GL-32 K, EMD Millipore), whereas concentrations of insulin and IGF-1 were measured by RIA as described earlier by Vicari et al. (2008).

Metabolites in blood such as glucose, NEFA, BHB, urea, cholesterol, and triglycerides were measured by an autoanalyzer (Cobas Mira, Roche) using commercial test kits (glucose: GLUC-PAP GL364; NEFA: NEFA FA115; BHB: RANBUT RB1007, from Randox Laboratories Ltd., and for Urea: UREA FS 1.3101 99 10 021, total cholesterol: CHOLESTEROL FS 1.1350 99 10 021, and trigylcerides: TRIGLYCERIDES FS 1.5760 99 10 021, from DiaSys Diagnostic Systems GmbH.)

For the measurements of urea and electrolytes in milk, milk serum was obtained by 2-step centrifugation at 1,900 × g for 15 min at 4°C and then at 20,800 × g for 30 min at 4°C. Milk urea was analyzed with a commercially available enzymatic kit (UREA FS 1.3101 99 10 021) from Randox Laboratories Ltd.). Electrolytes

Table 2. Chemical analysis of hay and concentrates[1]

Item[2]	Hay (n = 6)		AG (n = 4)		AL (n = 6)		Mixed (n = 5)		Glucogenic (n = 6)		Nitrogenic (n = 5)		Lipogenic (n = 6)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
DM (g/kg)	931	24.0	889	3.38	891	7.91	898	1.44	889	3.69	888	4.00	911	2.98
Energy (MJ NE_L/kg DM)	5.58	0.268	7.22	0.031	7.04	0.071	8.47	0.037	8.27	0.095	8.17	0.069	9.04	0.137
ADF (g/kg)	243	40.2	93.3	2.01	90	4.79	79.3	1.54	70.9	4.97	92.3	3.62	117	26.6
NDF (g/kg)	442	64.9	153	4.75	152	4.59	155	8.78	152	8.72	169	8.46	230	20.7
CA (g/kg)	95.76	11.5	95.4	5.11	136	5.49	41.6	0.748	30.6	0.304	57.3	2.95	38.4	1.16
CF (g/kg)	213	26	61	2.19	59.9	2.28	58.3	2.15	48.3	2.46	66.5	3.09	63.2	2.16
CL (g/kg)	ND		24.6	0.882	22.9	1.62	77.4	3.18	32.2	1.85	26.9	4.01	174	7.28
CP (g/kg)	141	12.4	334	4.02	319	4.74	224	4.35	161	5.43	332	21.7	170	3.59
Starch (g/kg)	ND		145	1.41	131	18.5	297	7.43	464	6.06	146	6.82	269	2.28
WSC (g/kg)	ND		69.9	2.73	65.4	10.9	56.1	2.69	58.3	22.8	70.6	3.94	47.9	2.42

[1]AG = compensation concentrate for dry cows; AL = compensation concentrate for lactation. AG and AL compositions are described in Table 1.
[2]CA = crude ash, CF = crude fiber, CL = crude lipid, WSC = water-soluble carbohydrates.

(Na, K, and Cl) were also measured with the autoanalyzer using ion-selective electrodes.

Statistical Analysis

Statistical analysis was performed by using the SAS software package (release 9.4; SAS Institute, Inc.). To test feeding effects the mixed models procedure (PROC MIXED) with the Tukey-Kramer adjustment for multiple comparisons was used. The model included time, dietary group, and interaction of time and feeding group (nitrogenic, glucogenic, lipogenic) as fixed effects, and cow as repeated subject and random effect. For evaluation of LPS effects, the Dunnett test procedure was used. Changes within dietary group were individually compared with the respective baseline value shortly before the LPS challenge (−1 or 0 h relative to LPS challenge) or the same time of day before LPS (−15 or −18h, respectively). Effects were considered to be significant when $P < 0.05$.

RESULTS

Effects of Feeding of Specific Nutrient Composition in the First Weeks of Lactation Before LPS Challenge

Milk Yield, BW, DMI, Energy Balance. Body weight did not significantly change throughout the 3 wk of feeding specific concentrates before LPS administration and was 701 ± 62 kg. In the third week of lactation, the DMI (Figure 1) was lower ($P = 0.03$ and 0.05, respectively) in glucogenic-fed cows compared with lipogenic and nitrogenic fed cows and was 19.1 ± 0.0, 22.2 ± 0.0, and 21.9 ± 0.9 kg in glucogenic, lipogenic, and nitrogenic fed cows, respectively. All cows were in a NEB with an energy balance of −51.3 ± 7.4 MJ NE_L/d in glucogenic-fed cows which was lower ($P = 0.03$ and 0.06, respectively) compared with −39.6 ± 9.1 MJ NE_L/d in lipogenic-fed cows and to −17.5 ± 9.4 MJ NE_L/d in nitrogenic fed cows.

Metabolic Parameters in Blood Plasma, Milk Composition. Metabolic parameters are shown in Figures 1, 2, and 3. The nitrogenic diet caused increased urea concentrations in blood and milk compared with glucogenic and lipogenic-fed cows at all time points ($P < 0.0001$, Figure 1). Plasma glucose, NEFA, and BHB concentrations were not affected by diet (Figures 1 and 2). Furthermore cholesterol, triglyceride, insulin, glucagon, and IGF-1 concentrations (Figures 2 and 3) in plasma was not affected by different diets in early lactation.

Milk SCC (Figure 4) tended to be lower in lipogenic-fed cows compared with nitrogenic fed cows ($P = 0.08$).

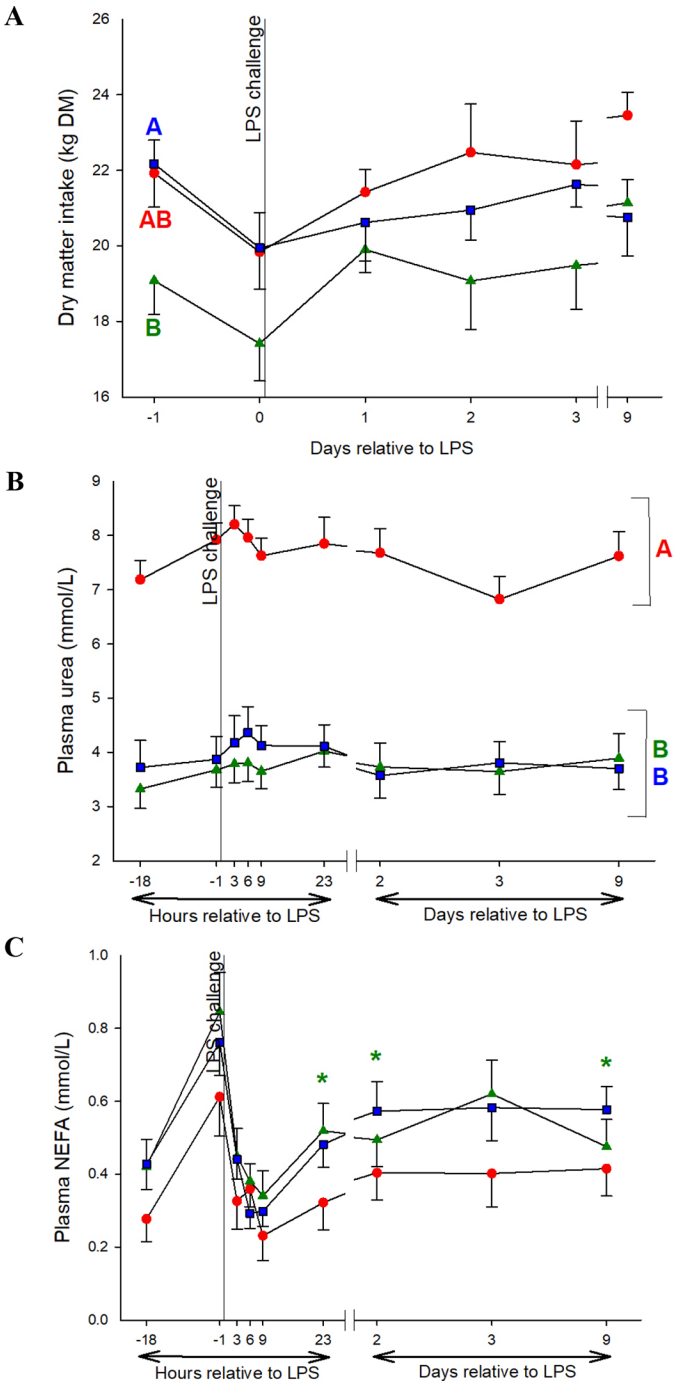

Figure 1. Dry matter intake (A), urea (B), and nonesterified fatty acids (NEFA; C) plasma concentrations in nitrogenic- (red circles), glucogenic- (green triangles), and lipogenic-fed cows (blue squares) before and after intramammary LPS challenge. Data are mean values ± SEM. Time relative to LPS indicates the time to the infusion of LPS into one quarter. A, B: Different letters indicate a significant difference ($P < 0.05$) for nitrogenic (red), glucogenic (green), or lipogenic (blue) group to the other treatment group within time point. * indicates a significant difference ($P < 0.05$) to the same time on the day before challenge (−1 h) within glucogenic-fed cows.

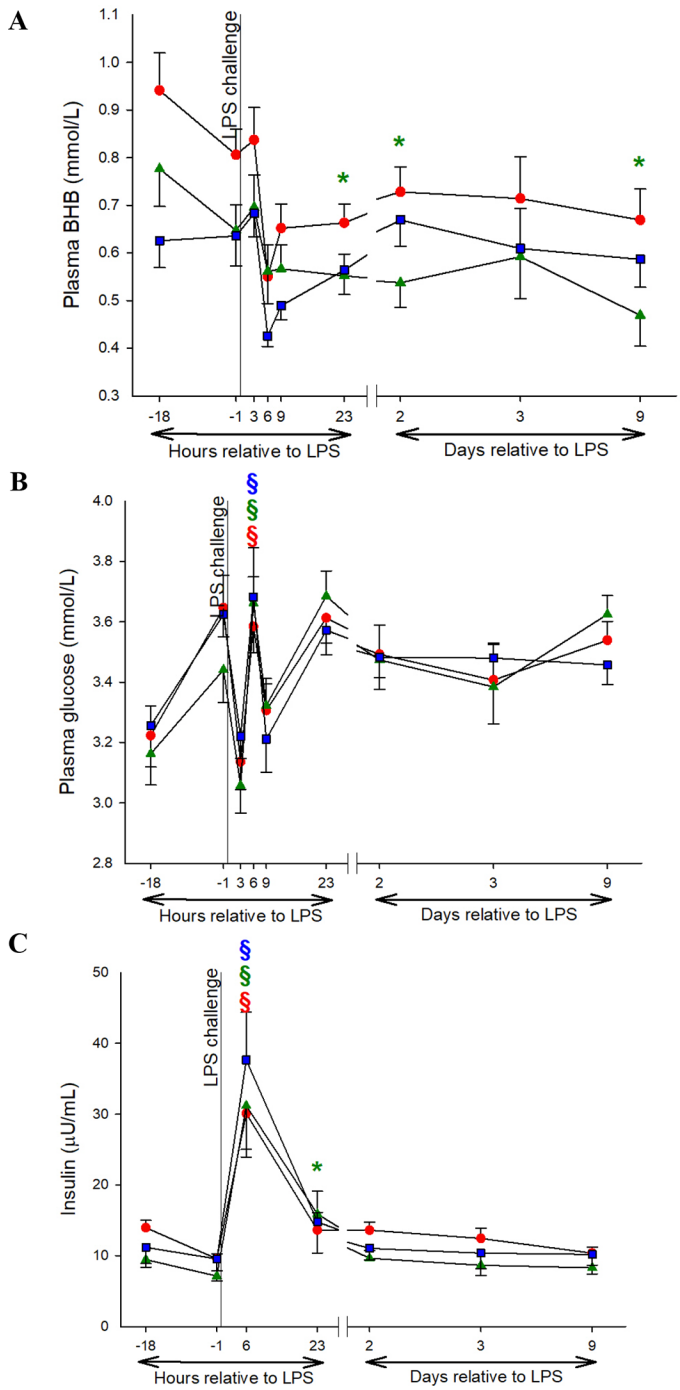

Figure 2. Plasma concentrations of BHB (A), glucose (B), and insulin (C) in nitrogenic- (red circles), glucogenic- (green rectangles), and lipogenic-fed cows (blue squares) before and after intramammary LPS challenge. Data are mean values ± SEM. Time relative to LPS indicates the time to the infusion of LPS into one quarter. * and § indicate a significant difference ($P < 0.05$) to the same time on the day before challenge (* to −1 h, and § to −18 h) within the nitrogenic (red), glucogenic (green), or lipogenic (blue) group.

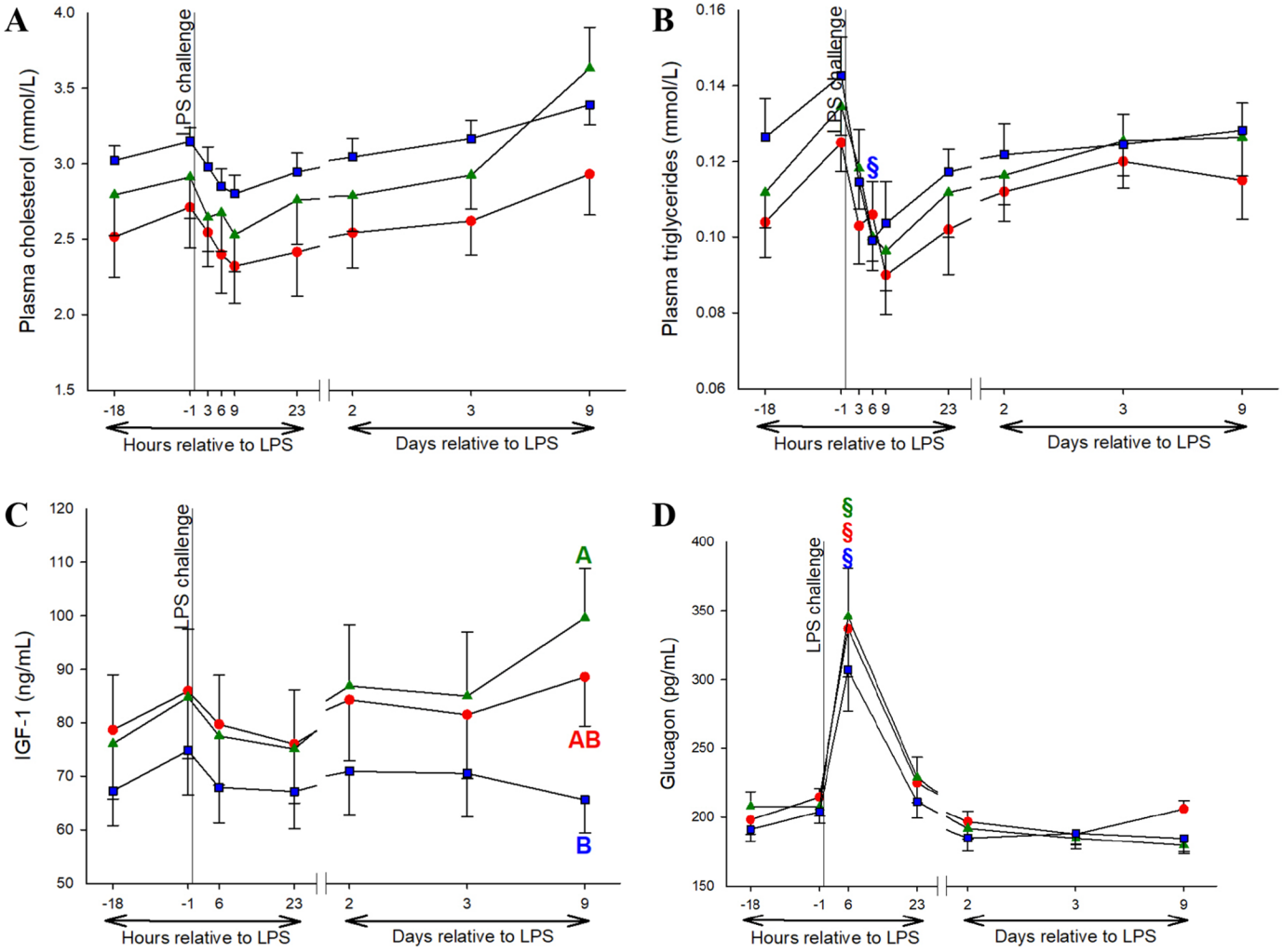

Figure 3. Cholesterol (A), triglyceride (B), IGF-1 (C), and glucagon (D) plasma concentrations in nitrogenic- (red circles), glucogenic- (green triangles), and lipogenic-fed cows (blue squares) before and after intramammary LPS challenge. Data are mean values ± SEM. Time relative to LPS indicates the time to the infusion of LPS into one quarter. A, B: Different letters indicate a significant difference ($P < 0.05$) between nitrogenic (red), glucogenic (green), or lipogenic (blue) group to other treatment groups within time point. § indicates a significant difference ($P < 0.05$) to the same time on the day before challenge (−18 h) within the nitrogenic, glucogenic, or lipogenic group.

Milk yield, milk fat content, and protein content (Figure 4) were not affected by diet.

Immunological Parameters in Blood. The WBC count, including lymphocyte and neutrophil granulocyte numbers (Figure 5) were not affected by diet. Furthermore, the phagocytic efficiency of blood leukocytes was not influenced (Figure 5).

LPS-Induced Mammary Inflammation in Early Lactation

Rectal temperature increased within 3 h after intramammary LPS challenge ($P < 0.05$, Figure 6) in all groups. A maximum was reached after 6 h in all groups and was already lower after 8 h compared with 6 h in all groups.

LPS-Challenged Quarter and Control Quarter for 9 h Hourly. Measured factors were not changed in hourly milk samples of control quarters (data not shown). Results of treated quarters are shown in Figure 6. In LPS quarters, SCC was elevated at time points 3 to 9 h compared with immediately before challenge in all groups ($P < 0.05$). Urea concentration in control quarters (data not shown), and LPS quarters was higher in nitrogenic fed cows compared with glucogenic and lipogenic-fed cows at all time points ($P < 0.05$) but was not affected by LPS challenge. Sodium concentration in LPS quarters was increased

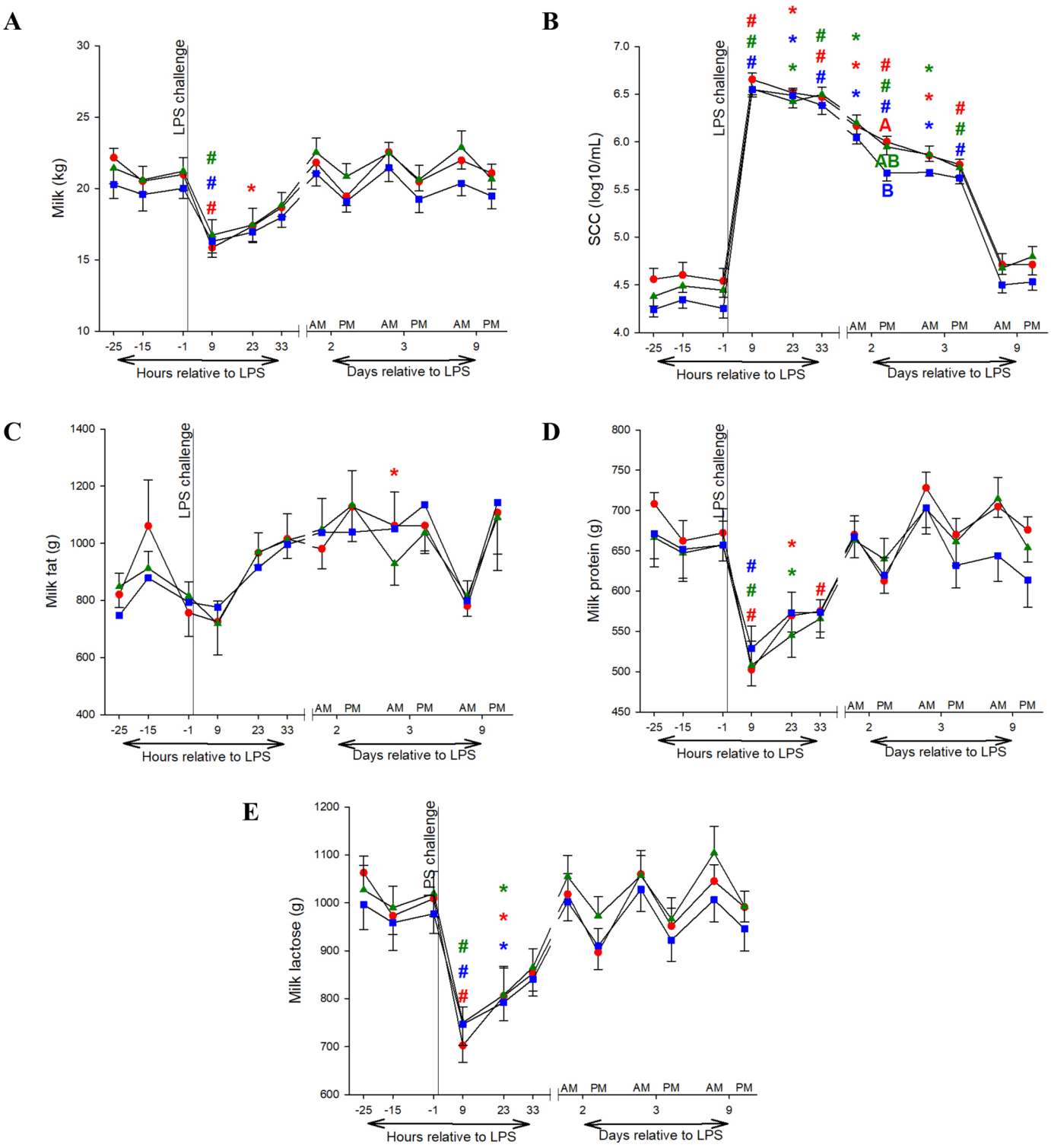

Figure 4. Milk yield (A), SCC (B), and fat (C), protein (D), and lactose (E) mass in milk in nitrogenic- (red circles), glucogenic- (green triangles), and lipogenic-fed cows (blue squares) before and after intramammary LPS challenge. Data are mean values ± SEM. Time relative to LPS indicates the time to the infusion of LPS into one quarter. A, B: Different letters indicate a significant difference ($P < 0.05$) for nitrogenic (red), glucogenic (green), or lipogenic (blue) group to the other treatment group within time point. * and # indicate a significant difference ($P < 0.05$) to the same time on the day before challenge (* to −1 h, and # to −15 h) within the nitrogenic, glucogenic, or lipogenic group.

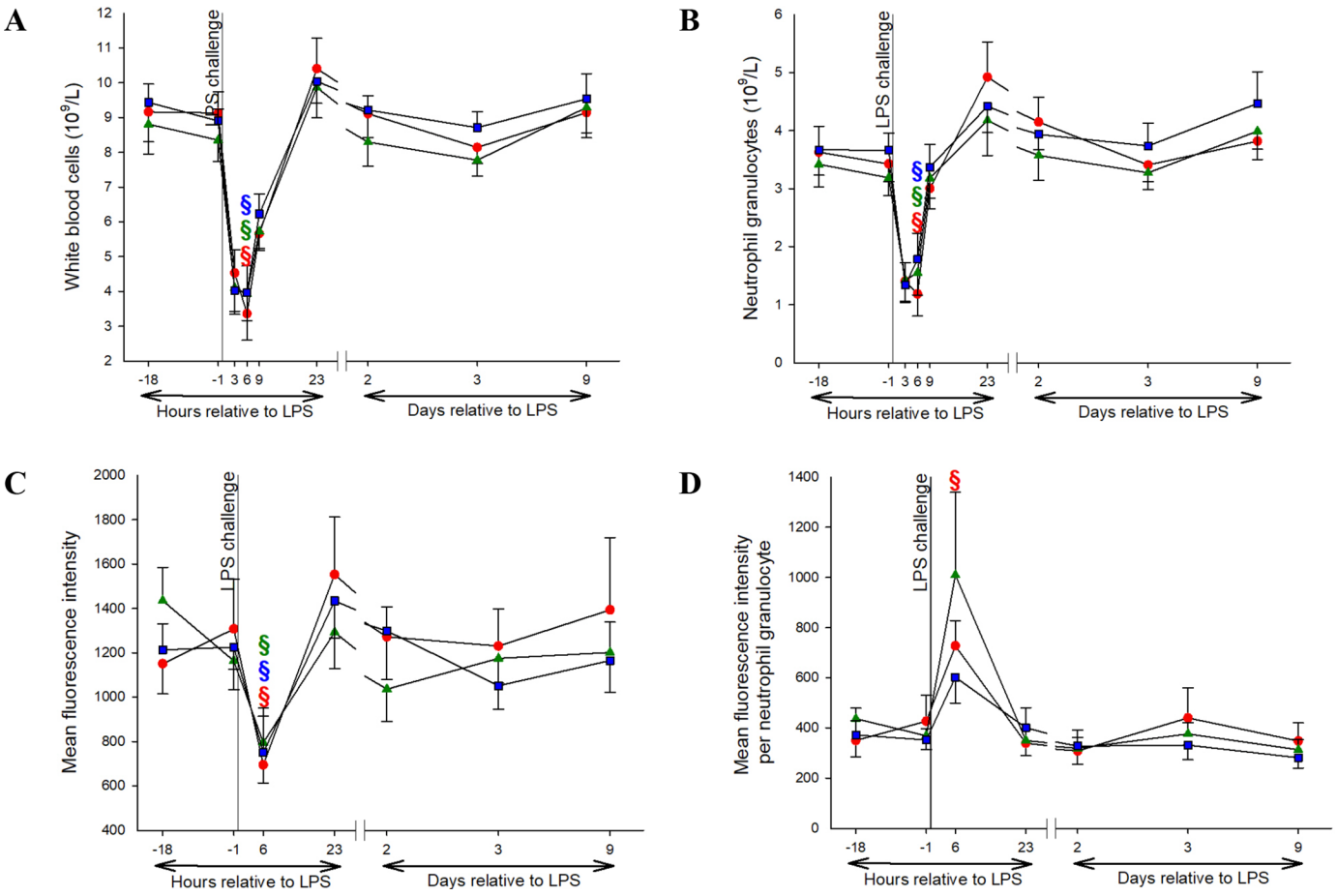

Figure 5. White blood cell count (A) and neutrophil (B) concentrations in blood, and total phagocytosis (C), and phagocytosis/neutrophil (D) in nitrogenic- (red circles), glucogenic- (green triangles), and lipogenic-fed cows (blue squares) before and after intramammary LPS challenge. Data are mean values ± SEM. Time relative to LPS indicates the time to the infusion of LPS into one quarter. § indicates a significant difference ($P < 0.05$) to the same time on the day before challenge (−18 h) within the nitrogenic (red), glucogenic (green), or lipogenic (blue) group.

at 3 h after challenge compared with 0 h in glucogenic and lipogenic-fed cows ($P < 0.05$) and at 4 h in all groups ($P < 0.05$). It remained elevated for all following hourly measurements. Chloride concentration in LPS quarters was elevated 4 h after LPS administration compared with 0 h in all groups ($P < 0.05$) and remained elevated for all following hourly measurements. Potassium concentration in LPS-challenged quarters declined within 3 h in glucogenic-fed cows ($P < 0.05$) and within 4 h in all groups.

Total Milk Around LPS Challenge. The total milk yield of the morning milking declined ($P < 0.05$) after LPS challenge in all groups compared with the morning milking before LPS challenge (Figure 4). On the morning after LPS challenge (23 h) only the milk yield of nitrogenic fed cows was still reduced compared with the morning immediately before LPS challenge but not in glucogenic and lipogenic-fed cows.

Milk fat concentration but not milk fat mass was elevated ($P < 0.05$) the morning after challenge in all groups (Figure 4). Milk protein concentration was not affected by LPS challenge. However, milk protein mass was lower ($P < 0.05$) the morning after LPS challenge compared with the milking before the LPS challenge in all cows (Figure 4).

Milk lactose concentration declined after LPS challenge in all groups and was lowest at 9 h ($P < 0.05$) in all groups; compared with the milking at the evening before LPS challenge (Figure 4). Milk lactose mass was reduced ($P < 0.05$) 9 h and 23 h after LPS challenge in all groups (Figure 4). LPS challenge had no effect on urea concentration and mass of urea in total milk of all cows.

Sodium concentration in total milk was higher the next day after LPS challenge in all groups ($P < 0.05$), whereas chloride concentration increased only in milk of cows fed glucogenic ($P < 0.05$) and lipogenic ($P < 0.05$) diets. Potassium concentration was not affected by LPS challenge on the day after challenge (data not shown).

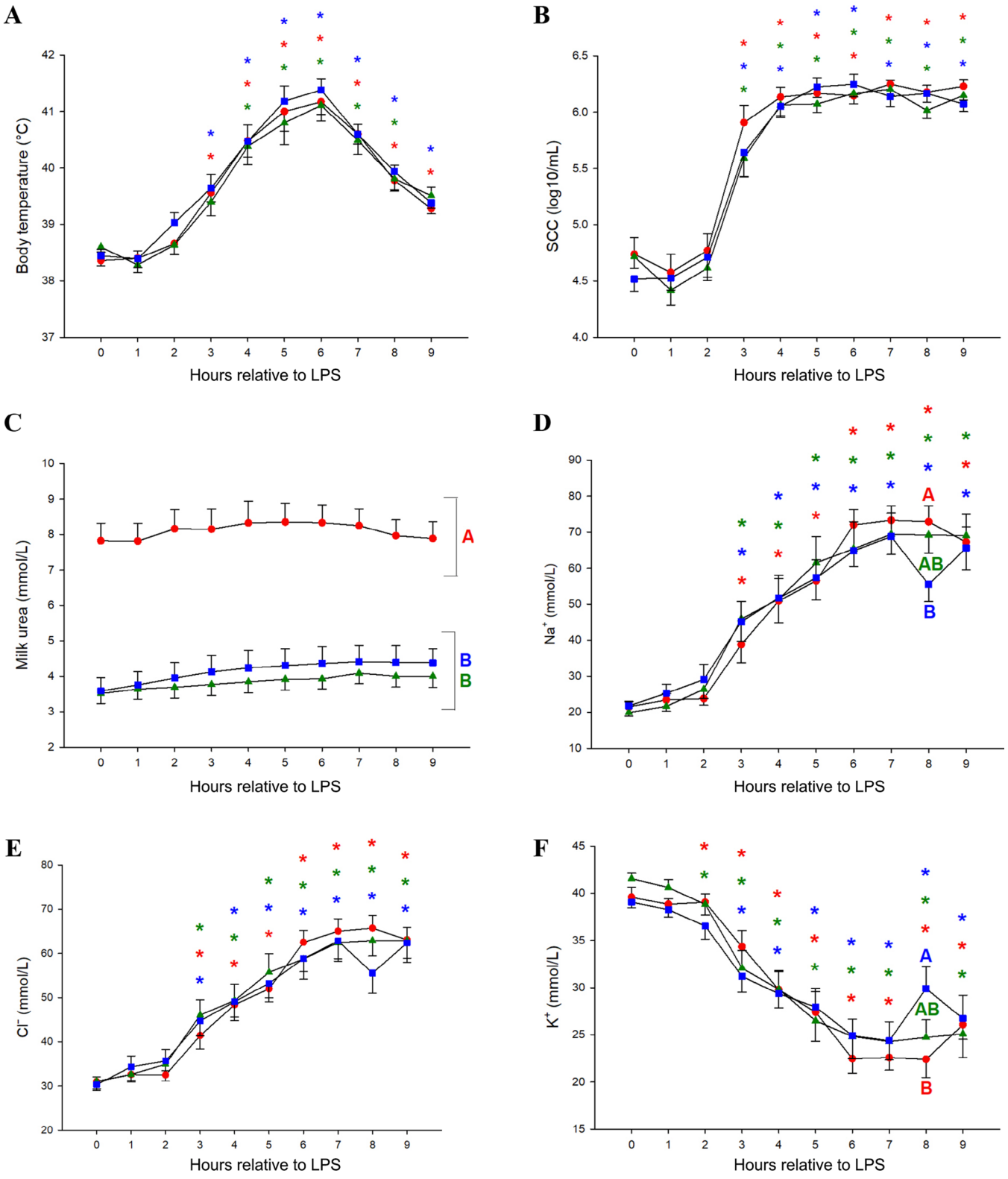

Figure 6. Hourly measured rectal temperature (A), and SCC (B), urea (C), sodium (D), chloride (E), and potassium concentrations in milk in LPS-challenged quarters in nitrogenic- (red circles), glucogenic- (green triangles), and lipogenic-fed cows (blue squares) on d 0. Data are mean values ± SEM. Time relative to LPS indicates the time to the infusion of LPS into one quarter. A, B: Different letters indicate a significant difference ($P < 0.05$) for nitrogenic (red), glucogenic (green), or lipogenic (blue) group. * indicates a significant difference ($P < 0.05$) to the same time on the day before challenge (−1 h) within the nitrogenic, glucogenic, or lipogenic group.

Immunological Parameters in Blood. The WBC count decreased ($P < 0.05$) after LPS challenge reaching a minimum at 6 h after challenge in all groups compared with −1 h (Figure 5). The WBC count was recovered ($P < 0.05$) again the next morning in all groups. Whereas lymphocyte numbers reached a minimum 6 h after challenge in all groups (data not shown), neutrophil numbers reached a minimum at 3 h in glucogenic and lipogenic-fed cows (Figure 5). Absolute phagocytosis was reduced ($P < 0.05$) at 6 h after LPS challenge in all cows (Figure 5). Phagocytosis per neutrophil was higher ($P < 0.05$) at 6 h after LPS challenge compared with −18 h in glucogenic-fed cows (Figure 5).

Metabolic Parameters in Blood. The LPS challenge of one quarter in the fourth week of lactation induced an insulin resistance indicated by increased ($P < 0.05$) insulin concentration at 6 h after LPS challenge and was still elevated in glucogenic-fed cows after 23 h (Figure 2). In addition, glucagon was increased ($P < 0.05$) in all groups at 6 h after challenge (Figure 3). Glucose concentrations were increased ($P < 0.05$) at 6 h after LPS challenge in all groups compared with the same time of day before LPS challenge (−18 h; Figure 2). Concentrations of NEFA and BHB (Figures 1 and 2) in plasma were decreased ($P < 0.05$) in glucogenic-fed cows on the day after LPS challenge. Cholesterol did not significantly change throughout the experiment in all groups (Figure 3). In addition, urea concentrations in plasma, DMI, and energy balance were not affected by LPS challenge in all groups (Figure 1). Solely triglycerides in plasma were reduced ($P < 0.05$) 6 h after LPS challenge in lipogenic cows compared with the same time of day before challenge but not in the other groups.

Plasma concentrations of IGF-1 were lower ($P < 0.05$) in lipogenic-fed cows than in glucogenic-fed cows on d 9 after LPS challenge (Figure 3).

Recovery of Mammary Inflammation

The characteristics of mastitis recovery were not influenced by dietary composition and all measured factors were recovered until 9 d after LPS challenge. After the initial decrease after LPS challenge, milk yield was recovered the second day after LPS challenge in all groups (Figure 4). The SCC was recovered 9 d after LPS challenge without differences between dietary groups (Figure 4). Milk protein, fat, and lactose concentrations and their total amounts were not different on the second day after LPS challenge until the end of the experiment compared with the milk before challenge without differences between dietary groups (Figure 4). The metabolic parameters glucose, insulin, glucagon, NEFA, BHB, cholesterol triglycerides, and urea in plasma were recovered 2 d after LPS challenge (Figures 1–3), except the NEFA and BHB concentration in glucogenic-fed cows.

DISCUSSION

Effects of Feeding of Specific Nutrient Composition in the First 3 Weeks of Lactation

Early lactation remains a critical phase in the life of dairy cows when significant changes in metabolism occur due to the onset of lactation and fast increasing milk production. It is well known that the risk of mammary infection is high during this period of catabolic metabolism. Therefore, this study aimed to investigate possibilities of manipulation of the metabolic status by feeding different concentrates that particularly provide specific macronutrients or respective precursors (i.e., proteins, carbohydrates, or lipids) before, during, and after undergoing a mammary inflammation in early lactation. All animals were in NEB during the entire experiment. Milk yield was not affected by the feeding strategies, likely because nutrients are directed to the mammary gland with a high priority at the onset of lactation and milk yield is maintained despite the NEB (reviewed by Gross and Bruckmaier, 2019).

The observed lower DMI during the glucogenic diet compared with a nitrogenic and lipogenic diet was comparable to cows in mid lactation receiving the same diet composition (Gross et al., 2021). Related to the reduced DMI, the energy deficiency was highest in the glucogenic-fed cows. According to Rastani et al. (2001), who compared performance parameters of Holstein and Jersey cows during the first 120 d in milk, the energy balance in Holstein cows can drop to −12.9 Mcal/d (i.e., −54 MJ/d) in wk 2 of lactation. In our studies only the glucogenic group was in a NEB similar to the nadir found by Rastani et al. (2001).

Metabolic parameters such as plasma glucose, insulin, glucagon, cholesterol, triglyceride, IGF-1, BHB, and NEFA concentrations were in the range expected in early lactation but they were not affected by the different diet. These findings are in contrast to the studies of van Knegsel et al. (2005, 2007), Studer et al. (1993), Minor et al. (1998), and Drackley et al. (2005). In these studies, feeding lipogenic nutrients was shown to increase plasma NEFA and BHB concentrations and led to decreased plasma glucose and insulin concentrations (van Knegsel et al., 2005). On the other hand, glucogenic nutrients had opposite effects and increased plasma glucose and insulin levels (van Knegsel et al., 2005, 2007) and decreased NEFA, BHB, and triglyceride levels (Studer et al., 1993; Minor et al., 1998;

Drackley et al., 2005). A possible explanation might be that, the diets did not exclusively provide CP, carbohydrates, or lipids, respectively, but concomitantly also other nutrients.

Increased urea concentrations in blood and milk of cows fed concentrate rich in high protein was expected and has already been observed by numerous other studies (Gross et al., 2021; reviewed by Cheng et al., 2015; Graber et al., 2012). Furthermore, it is known that a state of NEB impairs the liver function and therefore the metabolic clearance of urea (O'Callaghan et al., 2001).

The feeding strategies were obviously not sufficiently extreme to change the metabolism and elicit the NEB effects markedly. However, diets were selected based on realistic feeding strategies that could be easily applied in dairy farming. Furthermore, the cows were in NEB but healthy, therefore differences in the basic immune competence due to the different feeding strategies were not detectable based on WBC count, phagocytic capability or milk SCC. Hence an intramammary immune challenge was performed.

Effects of Intramammary LPS Challenge on Cows in Early Lactation Receiving Differently Composed Feed

Circulating plasma metabolites during NEB in early lactation have direct effects on immuno-competent cells in the mammary gland and milk (Suriyasathaporn et al., 1999). To our knowledge, this is the first study focusing on the direct comparison between nitrogenic, glucogenic, or lipogenic-accentuated feeding and their effects on the immune response during mammary inflammation in early lactation. The intramammary challenge of one quarter with LPS in our study induced the expected (Schmitz et al., 2004) local immune response shown by a substantial SCC increase within 4 h, whereas the SCC in the control quarter remained low. This was similar in all groups and show that the supply of different nutrients had no effect on the cell recruitment, which is crucial for the immune competence of the mammary gland and the course of mastitis (Mehrzad et al., 2005). The change of electrolytes in milk after LPS challenge (increase of Na^+ and Cl^- and decrease of K^+) within 3 to 4 h proved the impairment of the blood–milk barrier (Fernando et al., 1985; Caldeira et al., 2021) in our experiment. In control quarters the barrier remained intact. The blood–milk barrier integrity develops in the first days after parturition regulated by different hormones (reviewed by Wellnitz and Bruckmaier, 2021). In the third week of lactation, when the experiment was performed, the integrity of the barrier is already fully established and an impairment indicates an inflammatory reaction. Obviously, the impairment of the integrity of the blood–milk barrier during mammary inflammation was not affected by the dietary concentrate type.

The high urea concentrations in milk in nitrogenic fed cows, and also the low urea concentrations in glucogenic and lipogenic-fed cows were not influenced by LPS challenge. Urea can pass the epithelial barrier in both directions in the same extent throughout the entire mammary gland (Linzell and Peaker, 1971), and its distribution in the udder is, therefore, not influenced by the blood–milk barrier impairment during LPS challenge. The severity of the local inflammation in the mammary gland based on the SCC increase and opening of the blood–milk barrier, was obviously not influenced by the high urea concentrations. This agrees with Cheng et al. (2015) who showed that a high urea concentration does not alter the innate immune response. As an increased amount of urea in milk does not seem to have an influence on the growth of important mastitis pathogens (Stürmlin et al., 2021) it is also unlikely that higher urea concentrations as a result of a nitrogenic feeding would increase the severity of a bacterial infection of the mammary gland.

The LPS-induced inflammation of one quarter led to a decline in total milk yield in the morning on the day after LPS challenge. It is known that mastitis reduces the milk yield due to a reduced synthesis by mammary epithelial cells as a response to different immune factors such as cytokines (Shuster et al., 1991) and due to the energy shift toward the priority of the immune system and survival (reviewed by Bradford et al., 2015). However, milk yield during mastitis is also reduced due to the impairment of the blood–milk barrier through which lactose can transfer to the blood. Then its osmotic effect and, therefore, the water accumulation in the cisterns is reduced (Peaker, 1977). This decline in lactose concentrations in milk after LPS challenge was also seen in our study. Interestingly, the reduced milk yield during mammary inflammation was not influenced by the concentrate composition used in our experiment. This shows that this impairment of milk production by inflammation cannot be overcome by feeding specific nutrients such as the energy carrier glucose or lipids.

The impaired function of milk production during inflammation includes reduced protein and milk fat secretion (Harmon, 1994; Kitchen, 1981). Although the protein concentrations did not significantly change in our study, we could detect a reduction of protein mass on the day after challenge. On the other hand, milk fat concentrations increased one day after challenge but milk fat mass did not significantly change, which shows that the synthesis of milk fat did not change notice-

ably by LPS challenge without any difference between feeding groups. These effects were seen in total milk although mastitis was induced in our study only in one quarter. We assume that changes in only the challenged quarters would have been masked by dilution effects. Therefore, a reduction of milk production was likely induced in all quarters by systemic effects after inflammation of one quarter, which agrees with previous studies (Shuster et al., 1991).

Indeed, in addition to the local immune response, a systemically activated immune system in response to LPS challenge of one quarter was indicated by the increased rectal temperature. Furthermore, a short-term decrease of the phagocytic capacity in the blood was detected. This was expected as due to the tremendous increase of SCC in the challenged quarter the WBC concentration in the blood also decreases (Van Werven et al., 1997). The decrease included all kinds of leukocytes such as lymphocytes and different kinds of granulocytes (eosinophilic, basophilic, and neutrophilic). Interestingly, the phagocytic capacity of the single cell was increased during periods of neutropenia. The mammary immune capacity depends, among other things, on the number of circulating PMN at the time the trigger occurs (Heyneman et al., 1990) and on the rate of PMN diapedesis into the infected quarters (Hill, 1981; Vandeputte-Van Messom et al., 1993). Furthermore, the activity of bone marrow to provide young or active PMN (Van Merris et al., 2002; Burvenich et al., 2003), which is indicated by a fast recovery of WBC count after LPS challenge is decisive for an effective immune response. In our study, the WBC count was already recovered the next day without differences between dietary groups which indicates an active and not impaired immune response in all feeding groups independent of nitrogenic, glucogenic, or lipogenic feeding.

The feeding of different concentrates did not influence the decrease in plasma concentrations of NEFA after LPS challenge in our study. This decrease is in contrast to the study of Moyes et al. (2016) who detected an increased NEFA concentration in plasma during an *E. coli* mastitis in early lactation and discussed this with lipolysis for the energy requirement for the immune system. However, they also detected, similarly to our study, a decrease in BHB plasma concentration and attributed this to a loss into the milk through an impaired blood–milk barrier. In our study this cannot be the only reason, as BHB decreased only in glucogenic but not in nitrogenic- and lipogenic-fed cows, despite an impaired blood–milk barrier in all groups maybe due to a less pronounced NEB in nitrogenic- and in lipogenic-fed cows. In the study of Moyes et al. (2016) the authors also detected, a decrease in cholesterol and triglycerides, which was attributed to effects on the lipid metabolism by the inflammatory response (Moyes et al., 2016). This was not clearly seen in our study, where we could only detect a significant decrease of triglyceride in lipogenic-fed cows after LPS challenge. Although IGF-1 concentrations did not differ between groups before challenge, on d 9 after the LPS challenge the IGF-1 concentrations was lower in lipogenic-fed cows than in glucogenic-fed cows. As this growth factor is an epithelial mitogen (Forsyth, 1996), it is likely be involved in the tissue repair after LPS stimulation. Differences between feeding groups seem to be little and may be due to high variations as lipogenic-fed cows did not seem to have less severe mastitis based on the other measured factors (e.g., SCC on d 2, see below).

High levels of ketone bodies were shown to have a negative effect on the chemotactic activity of leukocytes in vitro (Suriyasathaporn et al., 1999). As ketone bodies were not significantly influenced by diet in our study, effects on SCC changes were not expected. Plasma concentrations of BHB decreased in glucogenic-fed cows significantly the day after LPS challenge but a difference in SCC increase to the other cows were not seen. Most likely the differences in BHB concentrations were not high enough to detect differences in the strong SCC increase.

The activated immune system needs glucose (Kvidera et al., 2017). However, we could not detect an increase of plasma glucose in the 3 feeding groups. Therefore, the constant phagocytic activity was expected and shows that the feeding of nitrogenic, glucogenic, or lipogenic feed in quantities that could be realized in practice may not influence the function of the cellular immunity.

An insulin resistance was shown before during LPS-induced mastitis in mid lactation (Vernay et al., 2012) and also in early lactation (Waldron et al., 2006). The induction of an insulin resistance and glycogenolysis and gluconeogenesis during mammary inflammation is induced to maximize glucose concentration in plasma to be available for the immune system (Vernay et al., 2012). In our study, in early lactation, there was also evidence of an insulin resistance (i.e., an increase in insulin concentration in plasma). Furthermore, glucose shortly increased in all cows after LPS challenge. During an inflammation in early lactation the provision of glucose for the immune system seems to be of particular importance as the energy balance in early lactation in dairy cows is often negative, which was also the case in our study. Therefore, the high glucose supply in glucogenic-fed cows was expected to positively influence the immune response, as an activated immune system needs a high amount of glucose as an energy source. We have shown earlier that experimentally induced hypoglycemia, as well as hyperketonemia, interact with the immune response during an LPS-induced mastitis

(Vernay et al., 2012; Zarrin et al., 2014a). However, we did not see any differences in neutrophil recruitment into the udder (i.e., SCC increase and WBC count decrease and recovery in the blood), between the different feeding groups and assume this effect of higher glucose supply by glucogenic feeding was not sufficient to detect differences in the mammary immune response.

Recovery of Mastitis in Cows in Early Lactation Receiving Differently Composed Feed

Circulating glucose and BHB concentrations were shown to be associated with the recovery of udder health (Gross et al., 2020). In the present study we investigated the recovery from mastitis in early lactation under different nutrient supply. It could be of great interest in practice if recovery from mastitis could be supported by specific feeding strategies. However, we could not see differences between the different feeding groups, indicating that neither a specific glucose supply through glucogenic feeding nor nitrogenic or lipogenic feeding had a considerable influence on the length of the mammary immune response. However, the study shows how fast the metabolism recovers from mastitis in early lactation. Metabolic changes that occurred by intramammary LPS challenge (i.e., increase of insulin, decrease of NEFA and BHB in glucogenic-fed cows and the short increase of glucose in all cows in plasma) were recovered the second day after immune challenge. In this experimentally induced mastitis it has to be taken into account that the challenge was performed only once and with milk production and milking, the concentration of applied LPS decreased constantly. However, the SCC was still elevated on the third day after challenge although the recruited cells into the mammary gland were removed twice a day during milking. Indeed Ziv et al. (1976) could detect LPS when infused in a high dosage (10 mg/quarter) in the udder after 48 h. The SCC was recovered to the initial value after 9 d without differences between feeding groups. Solely on the second day the SCC was slightly lower in lipogenic-fed cow. However, these little differences indicate that the trigger for SCC recruitment remains for several days, which was not substantially influenced by the different feeding strategies.

Milk production including milk fat and milk protein synthesis was recovered within 2 d after LPS challenge. As discussed before, the reduced milk production is induced by systemic mediators of inflammation, such as cytokines and glucocorticoids, during clinical mastitis (Shuster et al., 1991; Mitterhuemer et al., 2010), and it was shown before that glucocorticoid concentrations in plasma recover within 12 h after endotoxin mastitis (Huszenicza et al., 2004). Therefore, the results of the present study show that an endotoxin-induced mammary inflammation in early lactation of dairy cows leads only to a short-term disturbance of milk production, and its recovery is independent of a glucogenic, nitrogenic, or lipogenic-accentuated feeding.

CONCLUSIONS

The nitrogenic-, glucogenic-, or lipogenic-accentuated feeding in early lactation of dairy cows by the provided feed composition in this study seems to have only little effects on endocrine or metabolic response and milk production. Furthermore, based on the measured factors it seems that the immune response to a mammary inflammation induced by an intramammary LPS challenge, and the recovery of the mammary gland was not affected by these feed compositions. Therefore, the immune defense of dairy cows in the period of NEB in early lactation can, obviously, not sufficiently be supported via a specific diet rich in either CP, carbohydrates, or lipids in quantities that represent possible feed compositions on practical dairy farms.

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