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Metabolic and endocrine responses to short-term nutrient imbalances in the feed ration of mid-lactation dairy cows

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ABSTRACT

Short-term imbalances of dietary nutrients occur during natural fluctuations in roughage quality (e.g. on pasture) or temporal shortages of supplementary feed components. In contrast to a deficiency, macronutrients (i.e. carbohydrates, proteins, lipids) beyond the adequate supply with other nutrients may, for instance, alter milk composition, increase BW or result in a greater excretion of nitrogen. Especially dairy cows with a moderate performance, in mid- or late lactation, or in extensive farming systems may be exposed to imbalanced rations. A better understanding of metabolic and endocrine responses depending on macronutrient supply may help to precisely feed dairy cows. The present study investigated short-term metabolic and endocrine responses to different levels of concentrates formulated to particularly provide one major macronutrient source (carbohydrates, proteins or lipids). Based on parity number, lactational stage, milk yield and BW, nine mid-lactating cows (211 ± 19 days in milk) were grouped into three blocks of three animals each. Concentrates (aminogenic: rich in CP and nitrogen sources; glucogenic: high content of carbohydrates and glucogenic precursors; lipogenic: high lipid content) were fed in addition to hay in a factorial arrangement at increasing levels from 2.5 to 7.5 kg/d during 9 d. Milk yield, BW and feed intake were recorded daily. Blood and milk were sampled every 3 d at the end of each concentrate level. Milk fat, protein, lactose and urea contents were determined. In blood, concentrations of various metabolites, endocrine factors and enzyme activities (e.g. glucose, non-esterified fatty acids (**NEFAs**), β-hydroxybutyrate, urea, cholesterol, triglycerides, insulin, glucagon, aspartate aminotransferase (**ASAT**), gamma-glutamyltransferase (**GGT**) and glutamate dehydrogenase activity (**GLDH**)) were measured. Milk yield, milk composition and BW were not affected by type and level of concentrates. Feed intake increased in cows with greater amounts of the aminogenic and lipogenic concentrate compared with the glucogenic concentrate. Milk and plasma urea concentrations were elevated in the aminogenic and to a lesser extent in the lipogenic treatment compared with the glucogenic treatment. Glucose concentrations in plasma were not affected by treatments, whereas insulin and glucagon increased, and NEFA concentrations decreased only in cows fed 7.5 kg/d aminogenic concentrate compared with the glucogenic and lipogenic treatment. Activities of ASAT, GGT and GLDH as well as the total antioxidant capacity were not affected by diets. In conclusion, immediate metabolic and endocrine responses were observed due to the short-term dietary changes. Particularly, a surplus of nitrogen supply via the aminogenic diet affected metabolic responses and stimulated insulin and glucagon secretion.

Implications

Dairy cows with moderate performance, in advanced lactation or in extensive production systems, are likely to be fed above their energy and nutrient requirements. This study investigated the respective metabolic responses of mid-lactating cows to diets supplemented with increasing amounts of concentrates differing in their carbohydrate, protein and lipid content. Particularly when a surplus of nitrogen-rich feed was applied, insulin and glucagon secretion was stimulated. Physiological knowledge about the metabolic and endocrine responses to short-term alterations of dietary compositions is crucial to ensure a precise feeding and to optimize resource efficiency.

Introduction

Numerous physiological and environmental factors affect the metabolic status of dairy cows. Imbalances of nutrient supply are not only due to low feed intake as observed in the periparturient

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period or heat stress conditions (Gorniak et al., 2014). Likewise, imbalances of dietary nutrient contents occur due to fluctuations of feed quality (e.g. when cows are fed on pasture) or limited availability (e.g. ingredients for concentrates). Especially dairy cows with a moderate performance, in mid- or late lactation, or in extensive farming systems may be exposed to imbalanced rations (Garga et al., 2013). The dairy cow has different strategies to cope with such stressors predominantly depending on the physiological stage. The prevailing high metabolic priority of milk production following gestation during the first weeks and months of lactation manifests in the presence of reduced insulin sensitivity in peripheral tissues to support nutrient fluxes to the mammary gland (Gross and Bruckmaier, 2019; Karis et al., 2020). A considerable breakdown of body tissues occurs to maintain milk production even at the expense of developing metabolic diseases. Irrespective of the lactational stage, inflammatory processes trigger the competition for nutrients and favor the immune system at the expense of performance (Ingvartsen et al., 2003). At later lactational stages, shortfalls in nutrition are immediately accompanied by a reduced lactation performance, whereas animal health seems not to be compromised (Gross et al., 2011).

On the one hand, an additional supplementation of nutrients in form of concentrates may help to compensate deficiencies of basal roughage diets. Hence, energy- and protein-rich concentrates are primarily fed during the early lactation period considering the nutrient requirements of ruminants, whereas diets supplemented with fat ensure energy intake when cows are facing heat stress and consequently reduced feed intake (Grum et al., 1996; Patton et al., 2004). On the other hand, concentrate feeding can exert targeted effects, e.g. modifying milk fatty acid profile by feeding of different dietary fat sources, balancing of nutrients or reduction of emissions (Hook et al., 2011; Borreani et al., 2013). The formulation of diets and particularly that of concentrate composition does not only aim at meeting the requirements of farm animals. Additional beneficial effects were observed in terms of a greater feed intake, improved carcass quality and animal health (Verdú et al., 2015). By feeding a glucogenic diet during the transition and early lactation, energy balance improved along with a reduction of plasma β-hydroxybutyrate (**BHB**) and liver triglyceride (**TAG**) concentration (van Knegsel et al., 2007).

In the present study, we investigated the short-term metabolic effects of different levels of concentrate formulated to particularly provide one major macronutrient source (i.e., carbohydrates, proteins, lipids) in mid-lactating dairy cows. Dietary formulation aimed not at providing extreme diets, but practical approaches to provide a relatively high amount of selected macronutrients. The objectives of the present study were to induce transient changes in metabolism and insulin responsiveness through a differential supply of macronutrients by concentrate feeding. Endocrine alterations of the insulin and glucagon system were hypothesized to respond differently to increased levels of aminogenic, glucogenic and lipogenic concentrate.

Material and methods

Experimental design, animals and housing

The experimental procedures were in accordance with the Swiss law on animal protection and welfare, and were approved by the responsible cantonal committee of animal experimentation (approval no. 2018_35_FR).

Nine lactating cows were selected from the dairy herd of the Agroscope Research Station (Posieux, Switzerland) and were kept in a straw and sawdust-bedded tie-stall barn during the experimental period. The experiment was carried out in a 3 × 3 factorial arrangement. Factors were (1) diet represented by three different concentrate supplements, and (2) level of concentrate supplementation with three increasing levels each. Based on parity number, lactational stage, milk yield and BW, cows were grouped into three blocks of three animals each. All dairy cows (six Red Holstein, three Holstein-Friesian) were multiparous (parity no. 4.0 ± 1.4 [mean ± SD], range (from 2nd to 7th lactation), non- or early-pregnant, in their mid-lactation (211 ± 19 DIM at start of experiment) with a daily milk yield of 25.4 ± 3.6 kg (3-d average of 3 d immediately before the experiment), and had a BW of 701 ± 60 kg. At the onset of the experiment, cows were clinically examined by a veterinarian. Cows were twice daily transferred to an external milking parlor for milking (0600 and 1700 h), where milk yield and BW were automatically recorded.

Feeding regimen, diets and feed analysis

Diet formulation followed the recommendations of Agroscope (2016). Throughout the experiment, all cows received hay for ad libitum intake and additional 1.5 kg basal concentrate (BASAL; as fed) independent of the treatment concentrates. The order of treatments was randomly assigned to the blocks of animals. Treatments consisted of three types of concentrate supplements diverging in their major macronutrient composition: glucogenic (high content of carbohydrates and providing glucogenic precursors), lipogenic (high lipid concent), and aminogenic (rich in CP and providing nitrogen sources). Each type of concentrate was fed for 3 d at a constant level of 2.5 kg/d (as fed), and the level of concentrate supplementation was increased after 3 and 6 d to 5.0 and 7.5 kg/d, respectively. In total, each cow received one type of treatment concentrate continuously for 9 d. Before the start of the experiment and between treatments (i.e. switch of diet types), a standard concentrate (**STD**) was fed at a constant rate of 2.5 kg/d for 5 d. Concentrates were provided in equal portions three times daily. Intakes of hay and concentrates were measured daily considering respective residues. Feed samples of hay and the concentrates were analyzed for contents of DM, crude fat, CP, fiber (ADF and NDF), starch, water soluble carbohydrates and ash as described earlier by Heublein et al. (2017). Energy content and available protein content at the duodenum (**APD**) of the feeds were calculated based on the equations provided by Agroscope (2016). The nutrient and chemical composition of the experimental diets and concentrates are shown in Table 1.

Sampling and measurements in milk and blood

Milk and blood samples were obtained before and at the end of the respective 3-d lasting intervals per concentrate type and level. Milk samples were taken from the morning milking at 0600 h. Milk samples were mixed gently using a mixing device (Reax 20, Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) and aliquots stored at −80 °C until analysis. One aliquot of fresh milk (50 mL + Bronopol as preservative) was immediately analyzed for gross composition via an infrared analyzer (MilkoScan-FT, Fossomatic, Hillerød, Denmark) in the laboratory of Suisselab AG (Zollikofen, Switzerland). After the morning milking and prior to feeding, blood samples were obtained via puncture of the coccygeal vein into evacuated tubes (Vacuette, 9 mL with K_3EDTA, cat. no. 455036; Vacuette, 9 mL with serum clot activator, cat. no. 455092; Greiner Bio-One International GmbH, Kremsmünster, Austria). EDTA tubes were immediately cooled on wet ice until centrifugation at 2 000*g* (+4 °C, 20 min), whereas serum tubes were kept at room temperature for 6 h before being centrifuged. The harvested plasma and serum were stored in 1.5 mL snap-vial tubes at −80 °C until analysis.

Table 1
Nutrient and chemical composition (g/kg of DM, unless noted) of hay and concentrates used in the feeding experiment with dairy cows.

	Hay	Concentrate				
Item		BASAL	STD	Aminogenic	Glucogenic	Lipogenic
Dietary composition						
Barley		20.2	26.1	10.0	10.0	19.5
Wheat			24.8		19.9	
Corn kernels			24.8	10.0	39.8	19.5
Soybean meal		44.9	12.0	54.8	10.0	9.7
Rapeseed meal		24.7		15.0	10.1	9.9
Corn gluten			5.0			
Potato protein			2.0			
Molasses			3.4			
Rapeseed cake						10.1
Rumen protected fat						16.4
Dried sugar beet pulp				10.2	10.2	14.9
$CaCO_3$		5.0	1.4			
MgO		1.3				
NaCl		2.7	0.3			
Mineral-vitamin-premix[1]		1.2	0.2			
Chemical composition						
DM (g/kg)	880	903	874	895	893	922
Ash	104	141	39.8	52.4	28.4	35.7
CP	148	306	188	320	144	165
APD	86.1	167	122	198	106	94.0
Crude fat	n.d.	22.5	28.4	26.1	28.9	182
Crude fiber	222	72.5	24.8	79.4	55.1	68.9
ADF	263	106	n.d.	113	70.7	103
NDF	434	168	n.d	183	148	187
Starch	n.d.	129	n.d	158	479	283
WSC	n.d.	52.1	n.d.	71.8	44.3	44.3
Net energy (MJ/kg of DM)	5.1	6.6	7.1	7.6	8.1	9.1

Abbreviations: STD = standard concentrate; APD = available CP at the duodenum; WSC = water soluble carbohydrates.
[1] Premix containing per kg: 17 000 000 IE vitamin A, 1 350 000 vitamin D_3, 80 000 mg vitamin E, 12 000 mg Cu, 100 000 mg Zn, 1 450 mg I, 20 000 mg Mn, 750 mg Se, 500 mg Co.

In plasma, concentrations of glucose, non-esterified fatty acids (**NEFAs**), BHB, urea, TAG and cholesterol were measured with an autoanalyzer (Cobas Mira, Roche, Switzerland) using commercial enzymatic kits (kits from Randox Laboratories Ltd. (Switzerland), Schwyz, Switzerland; glucose: GLUC-PAP GL364; NEFA: NEFA FA115; BHB: RANBUT RB1007; kits from DiaSys Diagnostic Systems GmbH, Holzheim, Germany; urea: DiaSys Urea FS 1 3101 99 10 021; triglycerides: DiaSys Triglycerides FS 1 5760 99 10 021; cholesterol: DiaSys Cholesterol FS 1 1350 99 10 021). Activities of aspartate aminotransferase (**ASAT**), gamma-glutamyltransferase (**GGT**) and glutamate dehydrogenase activity (**GLDH**) were determined in serum using commercially available kits from DiaSys Diagnostic Systems GmbH (Holzheim, Germany; ASAT: DiaSys ASAT FS (IFCC mod) 1 2601 99 10 021; GGT: DiaSys Gamma-GT FS 1 2801 99 10 021) and Randox Laboratories Ltd. (Schwyz, Switzerland; GLDH: Randox GLDH GL441). Insulin and IGF-1 concentrations in plasma were measured by RIA as described by Vicari et al. (2008) and glucagon by a commercially available RIA (cat. no. GL-32 K, EMD Millipore, Zug, Switzerland). Concentration of nitric oxide (NO) metabolites (nitrate NO_3^- + nitrite NO_2^- = NO_x^-) was measured in plasma based on the nitrate conversion by added nitrate reductase (EC 1.6.6.2) to nitrite with the latter being determined by the Griess reaction as described earlier (Kahl et al., 1997; Blum et al., 2001). The NO_2^- was measured in the absence of NO_3^- reductase and concentrations of NO_3^- were considered as the difference between NO_x^- and NO_2^- concentrations. The sensitivity of the assay was 3.125 μmol/L. The total antioxidant capacity (**TAC**) in serum was measured by a commercial kit according to the manufacturer's instructions (OxiSelect Total Antioxidant Capacity Assay, cat. no. STA-360, Cell Biolabs Inc., San Diego, CA, USA). The intra-assay CV was 3.47%; the inter-assay CV was 8.55%.

Calculations and statistical analysis

Energy balance of animals was calculated as difference between energy intake via feed and energy requirements (based on the amount and composition of milk, maintenance requirement as function of the metabolic BW; Agroscope, 2016). The calculation of the revised quantitative insulin sensitivity check index (RQUICKI) followed the equations based on plasma concentrations of glucose, NEFA and insulin as described earlier (Holtenius and Holtenius, 2007). In addition, we calculated the insulin:glucagon ratio (**IGR**) at a molar ratio. Statistical analysis was carried out using SAS (version 9.4, SAS Institute Inc., Cary, NC). The MIXED procedure was used to analyze data with blocks and block × treatment considered random effects. Statistical evaluation revealed that the effects of block and block × treatment were not significant. Within each treatment (aminogenic, glucogenic and lipogenic concentrate), a MIXED model with concentrate level (2.5, 5.0 and 7.5 kg/d) as fixed and cow as random effect was applied to investigate effects increasing supply of the different concentrates on animal performance, milk composition, and metabolic and endocrine factors in blood. Differences between supply levels of concentrates within each type were tested using the Tukey-Kramer procedure adjusting tests for multiple comparisons. Effects were considered significant at $P < 0.05$, while tendencies toward significance were assumed when $0.05 \leq P < 0.10$.

Results

Milk yield and milk composition, feed intake, BW and energy balance

Milk yield and BW were not affected by type and level of experimental concentrates ($P > 0.05$; Table 2). Contents of fat, protein

and lactose in milk did not change with type and level of the experimental concentrates (Table 2). Milk urea concentration clearly increased in parallel to urea concentration in blood with increasing aminogenic and, though to a lower extent, lipogenic concentrate supply ($P < 0.05$; Fig. 1A, B), but was not affected by the glucogenic concentrate. Except for the glucogenic concentrate, increasing levels of aminogenic and lipogenic concentrates resulted in an elevated DMI (Table 2). Concomitantly, energy balance improved with increasing levels of all concentrate types and was above the respective energy requirements of dairy cows (Table 2).

Metabolites and hormones

Glucose concentration in plasma was not affected by the different levels and types of experimental concentrates (Fig. 2A). Increasing levels of the experimental concentrates reduced NEFA concentrations in plasma (lower values ($P < 0.05$) at A5.0 and A7.5 of the aminogenic concentrate compared with STD; tendency towards lower values ($P < 0.10$) at L7.5 of the lipogenic concentrate compared with STD), but not in the glucogenic concentrate (Fig. 2B). Whereas plasma BHB concentration was not affected in cows fed the aminogenic and glucogenic concentrates, cows receiving a high level of lipogenic concentrate (L7.5) showed the lowest plasma BHB concentration ($P < 0.05$; Fig. 2C). Insulin concentrations increased with increasing amounts of aminogenic ($P < 0.05$ at A7.5) and glucogenic concentrate ($P < 0.10$ at G7.5; Fig. 3A), but did not alter in cows receiving the lipogenic diet. Likewise, plasma glucagon concentration increased with increasing levels of the aminogenic concentrate ($P < 0.05$; Fig. 3B), but did not change in cows receiving the glucogenic and lipogenic concentrates. No alterations of the insulin:glucagon ratio were observed with increasing concentrate levels (Fig. 3C). Whereas the RQUICKI did not change with increasing feeding levels of glucogenic and lipogenic concentrates, a tendency towards significance was detected when feeding the aminogenic concentrate ($P < 0.10$ for A5.0 vs. STD; Fig. 3D). Plasma IGF-1 concentrations were not affected by diet and feeding level (Table 2). Similar to urea concentrations in milk, urea concentration in plasma was increased at elevated aminogenic and lipogenic concentrate levels ($P < 0.05$; Fig. 1A), but was not altered when feeding the glucogenic diet. Cholesterol and TAG concentrations and activities of ASAT and GLDH in plasma did not change in response to the experimental concentrates ($P > 0.05$; Table 2). Concentrations of NO_x^- in all plasma samples were below the detection limit of 3.125 μmol/L. The total antioxidant capacity in serum was not affected by treatment and concentrate level (Table 2).

Discussion

We investigated the short-term metabolic and endocrine effects of increasing levels of aminogenic, glucogenic and lipogenic concentrates in mid-lactation dairy cows.

Effects of dietary treatments on feed intake, BW, energy balance, milk yield and milk composition

Commonly, the addition of concentrates increases digestibility and overall DMI. However, the duration of 9 d to feed different concentrates at increasing levels in the present study seems rather short to allow for an entire ruminal adaptation. In addition, concentrate types were switched after a short washout period. These particularities should be kept in mind when comparing our observations with other findings. In the present study, DMI increased only with increasing amounts of aminogenic and lipogenic concentrate, while the supplementation of glucogenic concentrate did not

Table 2
Milk yield and composition, BW, feed intake, blood metabolites and hormones in cows receiving different kinds and amounts of experimental concentrates. Data are mean values ± SEM. Different superscripts (a–c) within a dietary concentrate treatment indicate differences between concentrate levels ($P < 0.05$).

Item	Dietary concentrate treatment													Effect of concentrate treatment
	Aminogenic				Effect of concentrate level	Glucogenic			Effect of concentrate level	Lipogenic			Effect of concentrate level	
	STD	A2.5	A5.0	A7.5	*P*-value	STD	G2.5	G5.0	G7.5	*P*-value	STD	L2.5	L5.0	L7.5	*P*-value	*P*-value
Milk yield (kg/d)	24.4 ± 1.7	23.6 ± 1.4	24.2 ± 1.2	25.3 ± 1.4	0.8652	25.0 ± 1.6	24.7 ± 1.3	24.3 ± 1.3	24.5 ± 1.3	0.9871	24.1 ± 1.4	23.7 ± 1.4	24.6 ± 1.4	24.3 ± 1.2	0.9703	0.8923
Milk fat (%)	4.15 ± 0.15	4.15 ± 0.13	3.95 ± 0.08	4.09 ± 0.10	0.5882	4.03 ± 0.18	4.30 ± 0.17	4.25 ± 0.11	4.15 ± 0.06	0.5303	3.96 ± 0.11	4.07 ± 0.11	4.26 ± 0.16	4.22 ± 0.16	0.3953	0.5771
Milk protein (%)	3.84 ± 0.08	3.85 ± 0.10	3.87 ± 0.10	3.95 ± 0.11	0.8626	3.87 ± 0.09	3.87 ± 0.08	3.91 ± 0.09	3.98 ± 0.09	0.7973	3.85 ± 0.11	3.89 ± 0.10	3.89 ± 0.09	3.92 ± 0.07	0.9569	0.8894
Milk lactose (%)	4.56 ± 0.05	4.52 ± 0.04	4.52 ± 0.05	4.52 ± 0.07	0.9422	4.62 ± 0.04	4.56 ± 0.05	4.54 ± 0.03	4.58 ± 0.04	0.5429	4.48 ± 0.05	4.38 ± 0.12	4.53 ± 0.04	4.52 ± 0.04	0.4473	0.0479[1]
BW (kg)	714 ± 19	738 ± 20	742 ± 21	746 ± 19	0.9936	740 ± 19	741 ± 20	743 ± 19	745 ± 19	0.9972	740 ± 20	740 ± 20	739 ± 21	738 ± 20	0.9998	0.9676
DMI (kg/d)	21.2 ± 0.7[b]	22.2 ± 0.7[ab]	22.7 ± 0.7[ab]	23.4 ± 0.8[a]	0.2217	21.9 ± 0.9	21.4 ± 0.8	22.9 ± 0.7	22.7 ± 1.1	0.5989	20.9 ± 0.6[b]	20.4 ± 0.8[b]	22.7 ± 0.5[a]	23.6 ± 0.5[a]	0.0014	0.6945
Feeding level relative to energy requirement (%)	96.2 ± 4.7[b]	103.5 ± 4.9[ab]	109.5 ± 4.1[a]	112.0 ± 4.4[a]	0.0866	97.4 ± 3.6[b]	95.7 ± 3.5[b]	108.7 ± 2.9[ab]	113.4 ± 5.8[a]	0.0102	96.5 ± 3.6[c]	98.8 ± 4.7[c]	111.4 ± 3.5[b]	123.8 ± 4.0[a]	< 0.0001	0.5446
IGF-1 (ng/mL)	114.1 ± 5.4	109.2 ± 8.0	117.1 ± 7.4	118.3 ± 10.0	0.8495	106.4 ± 6.0	104.4 ± 5.9	114.8 ± 7.0	118.8 ± 6.7	0.3492	109.6 ± 6.3	110.7 ± 8.0	121.6 ± 11.0	116.6 ± 8.2	0.7364	0.7408
Cholesterol (mmol/L)	4.04 ± 0.20	3.86 ± 0.20	3.78 ± 0.21	3.62 ± 0.19	0.5288	4.18 ± 0.25	4.09 ± 0.25	3.99 ± 0.20	3.92 ± 0.21	0.8548	3.85 ± 0.26	3.86 ± 0.24	3.86 ± 0.23	3.88 ± 0.22	0.9999	0.3015
Triglycerides (mmol/L)	0.119 ± 0.008	0.118 ± 0.006	0.113 ± 0.009	0.110 ± 0.006	0.8309	0.124 ± 0.009	0.124 ± 0.009	0.126 ± 0.012	0.118 ± 0.010	0.9427	0.120 ± 0.006	0.123 ± 0.007	0.118 ± 0.005	0.112 ± 0.006	0.6283	0.3422
TAC (mM UAE)	0.196 ± 0.004	0.199 ± 0.003	0.196 ± 0.003	0.198 ± 0.003	0.8234	0.199 ± 0.005	0.195 ± 0.005	0.194 ± 0.004	0.196 ± 0.003	0.8499	0.202 ± 0.004	0.202 ± 0.004	0.198 ± 0.004	0.198 ± 0.004	0.7586	0.2859
ASAT (U/L)	89.8 ± 9.6	84.1 ± 8.2	84.6 ± 8.7	78.0 ± 6.8	0.8076	87.1 ± 9.1	88.8 ± 8.3	87.4 ± 9.5	80.2 ± 8.0	0.9011	83.8 ± 4.3	86.9 ± 6.1	87.7 ± 6.4	80.0 ± 6.1	0.7803	0.9324
GGT (U/L)	26.0 ± 1.8	25.9 ± 1.7	26.4 ± 1.4	25.6 ± 1.5	0.9829	26.4 ± 1.5	26.3 ± 1.5	25.6 ± 1.8	26.9 ± 1.7	0.9487	27.3 ± 1.9	26.9 ± 2.0	25.8 ± 1.4	26.4 ± 1.6	0.9350	0.8526
GLDH (U/L)	17.6 ± 3.0	15.8 ± 2.4	15.4 ± 2.3	12.9 ± 1.9	0.6066	18.2 ± 3.4	18.9 ± 3.3	17.3 ± 3.3	13.8 ± 2.2	0.6654	15.9 ± 1.7	17.1 ± 2.2	19.3 ± 2.9	15.5 ± 2.3	0.6420	0.5949

Abbreviations: DMI = DM intake; TAC = total antioxidant capacity; UAE = uric acid equivalent; ASAT = aspartate aminotransferase; GGT = gamma-glutamyltransferase; GLDH = glutamate dehydrogenase activity; STD = standard concentrate; A2.5 = aminogenic concentrate fed at 2.5 kg/d; A5.0 = aminogenic concentrate fed at 5.0 kg/d; A7.5 = aminogenic concentrate fed at 7.5 kg/d; G2.5 = glucogenic concentrate fed at 2.5 kg/d; G5.0 = glucogenic concentrate fed at 5.0 kg/d; G7.5 = glucogenic concentrate fed at 7.5 kg/d; L2.5 = lipogenic concentrate fed at 2.5 kg/d; L5.0 = lipogenic concentrate fed at 5.0 kg/d; L7.5 = lipogenic concentrate fed at 7.5 kg/d.
[1] Significant effect of dietary concentrate treatment between glucogenic and lipogenic concentrates ($P < 0.05$).

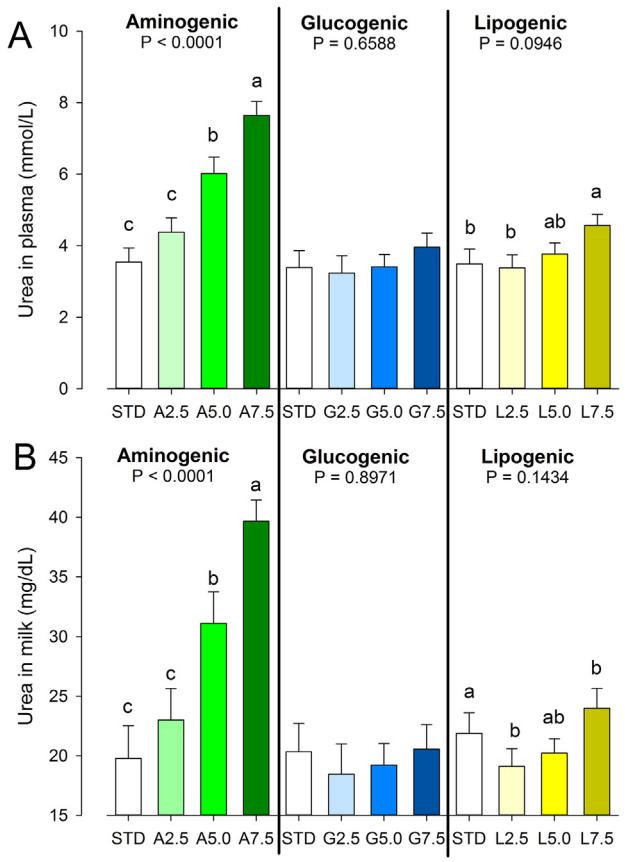

Fig. 1. Concentrations of urea in plasma (A) and milk (B) in dairy cows (n = 9) receiving different kinds and amounts of experimental concentrates. Data are mean values ± SEM. Different superscripts (a–c) within a dietary concentrate treatment indicate differences between concentrate levels (P < 0.05). Abbreviations: STD = standard concentrate; A2.5 = aminogenic concentrate fed at 2.5 kg/d; A5.0 = aminogenic concentrate fed at 5.0 kg/d; A7.5 = aminogenic concentrate fed at 7.5 kg/d; G2.5 = glucogenic concentrate fed at 2.5 kg/d; G5.0 = glucogenic concentrate fed at 5.0 kg/d; G7.5 = glucogenic concentrate fed at 7.5 kg/d; L2.5 = lipogenic concentrate fed at 2.5 kg/d; L5.0 = lipogenic concentrate fed at 5.0 kg/d; L7.5 = lipogenic concentrate fed at 7.5 kg/d.

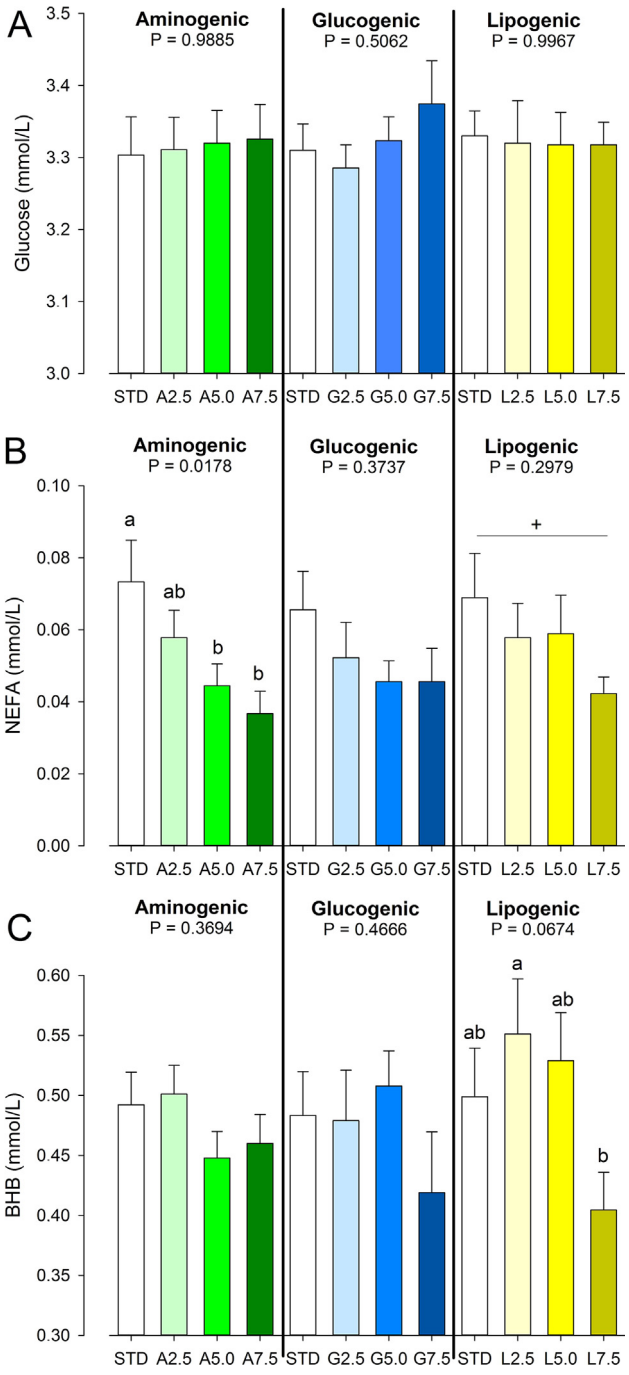

Fig. 2. Plasma concentrations of glucose (A), NEFA (B), BHB (C) in dairy cows (n = 9) receiving different kinds and amounts of experimental concentrates. Data are mean values ± SEM. Different superscripts (a–c) within a dietary concentrate treatment indicate differences between concentrate levels (P < 0.05). Tendencies towards significance are marked with "+". Abbreviations: NEFAs = non-esterified fatty acids; BHB = β-hydroxybutyrate; STD = standard concentrate; A2.5 = aminogenic concentrate fed at 2.5 kg/d; A5.0 = aminogenic concentrate fed at 5.0 kg/d; A7.5 = aminogenic concentrate fed at 7.5 kg/d; G2.5 = glucogenic concentrate fed at 2.5 kg/d; G5.0 = glucogenic concentrate fed at 5.0 kg/d; G7.5 = glucogenic concentrate fed at 7.5 kg/d; L2.5 = lipogenic concentrate fed at 2.5 kg/d; L5.0 = lipogenic concentrate fed at 5.0 kg/d; L7.5 = lipogenic concentrate fed at 7.5 kg/d.

affect DMI, although feeding level exceeded energy requirements in all treatments. Grum et al. (1996) observed a greater DMI when additional fat was added to a low concentrate diet, but a reduced DMI if fat was supplemented with a high concentrate diet. Confirming our results, fat supplementation at later lactational stages resulted in greater DMI irrespective of the basal energy content of diets (DePeters et al., 1989). In contrast to earlier reports without or depressing effects of dietary fat supplementation on DMI (e.g. Heinrichs et al., 1982; Beam and Butler, 1997; Moallem et al., 2000), the lipogenic concentrate in our study contained a considerable amount of cereals besides dietary fat sources. Even at the highest concentrate supplementation level, dietary fat content did not exceed 6% in the DM assuming that rumen fermentation and fiber digestion were not impaired. In contrast to an abomasal oil infusion, abomasal casein and starch infusions were shown not to alter DMI (Relling and Reynolds, 2008). Likely, postabsorptive effects of diets affecting appetite regulation might additionally contribute to different findings in terms of DMI (Relling and Reynolds, 2008).

In the present study, BW, milk yield, milk fat, protein and lactose were not affected by dietary treatments. We assume that the duration of the respective feeding periods was too short to induce major alterations in milk production and BW at this rather late lactational stage. Furthermore and in agreement with the present results, milk yield and milk fat content were not altered by abomasal infusions of oil, protein or starch (Aikman et al., 2002; Relling and Reynolds, 2008), although fat supplementation often

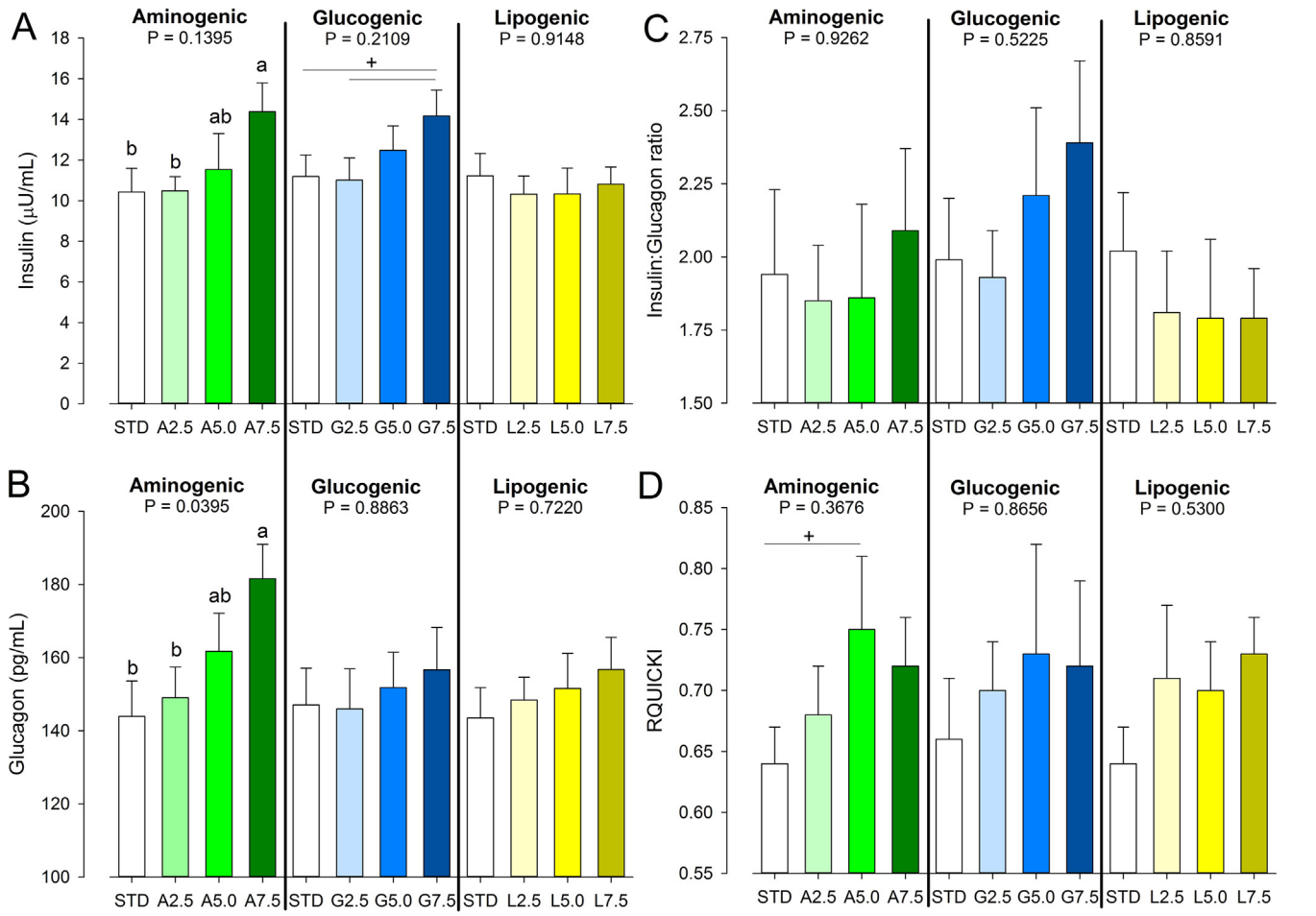

Fig. 3. Plasma concentrations of insulin (A) and glucagon (B), the insulin:glucagon ratio (C), and revised quantitative insulin sensitivity check index (RQUICKI; D) in dairy cows (n = 9) receiving different kinds and amounts of experimental concentrates. Data are mean values ± SEM. Different superscripts (a–c) within a dietary concentrate treatment indicate differences between concentrate levels (P < 0.05). Tendencies towards significance are marked with "+". Abbreviations: STD = standard concentrate; A2.5 = aminogenic concentrate fed at 2.5 kg/d; A5.0 = aminogenic concentrate fed at 5.0 kg/d; A7.5 = aminogenic concentrate fed at 7.5 kg/d; G2.5 = glucogenic concentrate fed at 2.5 kg/d; G5.0 = glucogenic concentrate fed at 5.0 kg/d; G7.5 = glucogenic concentrate fed at 7.5 kg/d; L2.5 = lipogenic concentrate fed at 2.5 kg/d; L5.0 = lipogenic concentrate fed at 5.0 kg/d; L7.5 = lipogenic concentrate fed at 7.5 kg/d.

increased milk fat content (Grum et al., 1996; van Knegsel et al., 2007). Lipogenic concentrate did not alter milk protein content in agreement with findings of van Knegsel et al. (2007) but increased milk urea content in the present study, whereas earlier research showed even a decline in milk protein content while milk urea was not affected (Grum et al., 1996). In contrast, milk protein content increased after abomasal casein infusion (Aikman et al., 2002; Relling and Reynolds, 2008). The most obvious increase in plasma and milk urea occurred in the aminogenic treatment. Elevated urea concentrations in plasma and milk are the result of excessive dietary nitrogen relative to energy supply exceeding the needs of rumen microbial protein synthesis (Nousiainen et al., 2004). Furthermore, urea formation and excretion are energy demanding processes. The glucogenic concentrate in the present study provided sufficient carbohydrates as energy source. Our lipogenic concentrate was rather low in its CP content and provided less glucogenic precursors compared with the glucogenic concentrate. Our observations on the elevated urea concentrations in the lipogenic treatment are in line with the findings of Kristensen et al. (2010), who found an increased arterial urea extraction across the rumen and portal-drained visceral tissues when cows were changed to a low nitrogen diet. In combination with a concomitantly lower carbohydrate supply, this might explain the increased urea concentrations in the lipogenic treatment.

Effects of dietary treatments on metabolic and endocrine responses

The effects of different macronutrients on metabolic and endocrine responses were primarily described in human literature with focus on physical exercise and type-2-diabetes, but less attention was paid so far to farm animals. Notably, physiological phenomena like insulin resistance related to human type-2-diabetes are not fully transferable to dairy cows as basal plasma glucose concentrations are markedly lower compared with humans.

Dietary treatments had no effect on plasma glucose concentrations in the present study. High concentrate diets with and without fat supplementation did not alter plasma glucose concentration either (Grum et al., 1996). Earlier research showed that in human subjects, the postprandial responses of plasma glucose, insulin and glucagon concentrations are related to meal compositions (Kawai et al., 1987). Concentrations of glucose and insulin in plasma increased to a greater extent in healthy subjects exposed to an oral glucose load, while responses were lowest after a protein-rich meal (Kawai et al., 1987). Besides insulin, particularly glucagon is involved in the regulation of glucose homeostasis (Unger and Orci, 1976). Insulin is acknowledged to act as a suppressor of glucagon secretion (Bansal and Wang, 2008), whereby the concomitant glucose turnover rate seems to be conjointly decisive if glucagon concentration increases or decreases (Zarrin et al., 2015). Hyperglycemia and hyperlipidacidemia inhibit glucagon

secretion (Unger et al., 1969), confirming our present results for the glucogenic and lipogenic treatments. Concomitantly, plasma glucagon concentrations decreased after glucose administration and increased after the consumption of a high-fat or high-protein meal (Kawai et al., 1987). Commonly, insulin and glucagon are counterregulated. Aminogenic diets contributing to elevated plasma amino acid concentrations are known to stimulate glucagon secretion (Unger et al., 1969). Particularly, diets rich in CP and poor in carbohydrate content provoke this glucagon response, as the secondary rise of insulin concentration would further lower the availability of glucose (Unger et al., 1969). Thus, an elevated glucagon concentration aims to prevent the decline in plasma glucose concentrations secondary to the concomitantly induced insulin secretion. Similarly, the infusion of insulin in dairy cows inducing hypoglycemia increased glucagon concentration, while reduced glucagon concentrations were observed during euglycemia with glucose concentrations maintained (Zarrin et al., 2015). Therefore, insulin and glucagon do not have to respond inversely.

Results on responses of IGF-1 to different dietary compositions are controversial. However, our present study was carried out in multiparous cows in mid-lactation, where the growth hormone-IGF-1 axis functions differently as compared with early lactation. Furthermore, parity number and energy status play a role in the response of insulin and the somatotropic axis. In the study by Grum et al. (1996), fat feeding with and without high amounts of grain decreased IGF-1, whereas Ronge et al. (1988) observed an elevated plasma IGF-1 concentration compared with an isocaloric diet without fat supplementation. Similar to IGF-1, Grum et al. (1996) showed a tendency towards a reduction of insulin concentration in the plasma of fat supplemented cows, while high grain diets increased plasma insulin concentration. Before parturition and during early lactation, insulin concentration in primiparous cows was not affected by dietary treatments, but was higher in multiparous cows fed a glycogenic compared with a lipogenic diet (van Knegsel et al., 2007). An increase in insulin concentration enables the nutrient partitioning into adipose tissue as lipolysis is concomitantly reduced. Insulin concentrations increased with elevating amounts of aminogenic and glucogenic concentrate, but not in the lipogenic treatment. Similar to our findings, Relling and Reynolds (2008) detected no alterations in plasma glucose concentrations despite changes in plasma insulin concentrations. Independent of the lactational stage, we could show in an earlier study that intravenous glucose infusions increased insulin concentrations while plasma NEFA and BHB concentrations concomitantly declined (Grossen-Rösti et al., 2018).

Similarly, glucogenic diets reduced plasma NEFA and BHB concentrations compared with lipogenic diets assuming a reduced energy partitioning towards milk fat production as indicated by a lower milk fat content and lipolysis of adipose tissue (van Knegsel et al., 2007). In another study, plasma BHB concentration was not altered by fat supplementation, but tended to be declined in cows receiving a high concentrate diet (Grum et al., 1996).

Elevated plasma lipid and protein concentrations trigger the secretion of insulin to remove abundantly available nutrients from circulation (Sano et al., 1995). The greater CP content of the aminogenic concentrate in our study implies an elevated supply with amino acids. Amino acids may act as secretagogues for insulin prior to the adaptations of the urea cycle and amino acid catabolism in response to the increased CP uptake (Relling and Reynolds, 2008). The latter clearly manifests in increased urea concentrations in plasma and milk in the present study. In contrast to our findings where plasma insulin concentration did not change in cows receiving the lipogenic diet, insulin concentration declined in studies with abomasal oil infusion or other dietary fat supplementation (Relling and Reynolds, 2008). Whereas the lipogenic concentrate fed to dairy cows in the present study contained also a considerable amount of starch, the rather pure fat supplementation in the latter cited research articles was accompanied by a lower DMI and consequently less absorbed nutrients stimulating insulin secretion. In contrast to our findings, high-fat overfeeding in humans resulted in elevated postprandial glucose and insulin concentrations (Parry et al., 2017). Similar to our study, where plasma NEFA concentration tended to decrease with increasing amounts of lipogenic concentrate, NEFA concentrations declined after high-fat overfeeding indicating a suppression of lipolysis of adipose tissue along with lowered plasma triglyceride concentrations assuming a respective lower endogenous production (Parry et al., 2017). Interestingly, plasma cholesterol concentrations here and in another experiment by Parry et al. (2017) were not affected by fat feeding. However, multiparous cows fed a lipogenic diet during the dry and early lactation period had elevated cholesterol concentrations compared with cows receiving a glucogenic ration (van Knegsel et al., 2007). In contrast, plasma NEFA concentrations increased following dietary fat supplementation or abomasal oil infusion (Grum et al., 1996; Relling and Reynolds, 2008). Parry et al. (2017) further hypothesized elevated NEFA concentrations to be involved in the development of insulin resistance and diabetes. However, the lacking effects of the lipogenic treatment on insulin and glucose concentrations in our experiment did not indicate an alteration of systemic insulin sensitivity as shown by the RQUICKI values. Changes in insulin signaling in response to caloric excess are likely to occur prior to increases in BW. Both high-carbohydrate and high-fat overfeeding for 5 days did not increase BW in humans, but induced changes in skeletal muscle insulin signaling before alterations in systemic insulin sensitivity became evident (Adochio et al., 2009). Likewise, we did not see changes in BW of dairy cows although feeding the different types of concentrates was above the energy and nutrient requirements. In the study of Adochio et al. (2009), the intramyocellular lipid content increased following the high-carbohydrate and high-fat overfeeding affecting therefore only insulin signaling in skeletal muscles but not overall body insulin sensitivity. We can only speculate about similar local effects of experimental treatments in dairy cows of the present study. However, the largely insulin resistant stage of peripheral tissues including adipose tissue as observed during early lactation (Karis et al., 2020) was not evident in the experimental period conducted in mid-lactation. Oxidative stress is greater in early lactation compared with later lactational stages (Cigliano et al., 2014). Greater hepatic lipid accumulation and increased concentrations of NEFA favor the production of reactive oxygen species and consequently oxidative stress (Turk et al., 2008). The total antioxidant capacity represents the protective capacity against oxidative damage and is greater in mid-lactation than in early lactation (Cigliano et al., 2014). The experimental diets in the present study showed a tendency to reduce plasma NEFA concentrations. Therefore, TAC levels were not negatively influenced. Despite the increased urea concentrations in plasma of cows receiving the aminogenic diet, we do not assume that cows in the present study developed a marked oxidative stress. In contrast, a long-term exposure to volatile basic nitrogen increased oxidative stress in dairy cows (Tsunoda et al., 2017). Though we did not determine amino acids in feed and blood, we expected a greater arginine supply in the aminogenic group that serves as substrate for the endogenous NO production (Blum et al., 2001). However, basal NO metabolites in dairy cows are low anyway and an activation of NO formation in healthy animals without infections can be excluded (Blum et al., 2001). Therefore, it is not surprising that NO_x- concentrations in plasma were not affected by dietary treatments.

Overall, the supply of aminogenic, glucogenic and lipogenic concentrate in mid-lactation dairy cows induced transient changes

in metabolism and insulin responsiveness. Contrary to our expectations, only the aminogenic treatment altered plasma insulin and glucagon concentrations. Due to the short duration of experimental treatments, animal performance was not affected. The extent of metabolic responses was rather low as all animals were in a positive energy balance, though particularly the surplus of nitrogen in the aminogenic diet affected metabolic adaptations.

Ethics approval

The experimental procedures were in accordance with the Swiss law on animal protection and welfare, and were approved by the responsible cantonal committee of animal experimentation (Canton of Fribourg, Switzerland, approval no. 2018_35_FR).

Data and model availability statement

None of the data were deposited in an official repository. Data are available from the corresponding author upon request.

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Declaration of interest

None.

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