

1 **Interpretive Summary:**

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3 **Effects of dry period energy intake on insulin resistance, metabolic adaptation, and production**
4 **responses in transition dairy cows on grass silage-based diets**

5 By Salin et al. Periparturient period is associated with insulin resistance in ruminants. Oversupply
6 of energy during the dry period may have adverse effects on metabolic adaptation, by affecting
7 maternal insulin resistance, accretion and mobilization of body reserves and feed intake of
8 periparturient dairy cows. Our study showed that free access to grass silage during the dry period
9 did not negatively affect metabolic changes or feed intake during the transition period compared
10 with controlling the energy intake by diluting grass silage with wheat straw.

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13 **ENERGY INTAKE AND INSULIN RESISTANCE IN COWS**

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15 **Effects of dry period energy intake on insulin resistance, metabolic adaptation, and production**
16 **responses in transition dairy cows on grass silage-based diets**

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27 **ABSTRACT**

28 High energy intake in the dry period has reportedly had adverse effects on mobilization of body
29 reserves, DMI, and productivity of dairy cows. We investigated whether grass silage (GS) fed ad
30 libitum (HEI; 141% of daily metabolizable energy (ME) requirements) in 8 wk dry period affects
31 metabolic adaptation and specifically peripheral insulin resistance, compared with ad libitum fed
32 total mixed ration (TMR) consisting of GS, wheat straw (WS) and rapeseed meal (55/40/5%; 108%
33 of ME/d; CEI). Multiparous Ayrshire dairy cows (n = 16) were used in a randomized complete block
34 design until 8 wk after parturition. Commercial concentrates were fed 1 and 2 kg/d during the last
35 10–6 and 5–0 d before the expected calving date, respectively. Postpartum, a similar lactation diet
36 with ad libitum access to GS and increasing concentrate allowance (max. 16 kg/d) was offered to
37 all. HEI group gained more body weight and had higher plasma insulin, glucose, and β -
38 hydroxybutyrate concentrations than CEI prepartum. Postpartal plasma glucose tended to be higher
39 and milk yield was greater from wk 5 onwards for HEI than CEI cows. Intravenous glucose tolerance
40 test (IVGTT) was performed at -13 ± 5 d and 9 ± 1 d relative to calving. HEI cows had greater
41 insulin response to glucose load and smaller area under the response curve for glucose than CEI
42 cows in prepartal IVGTT. Thus, compensatory insulin secretion adapted to changes in insulin
43 sensitivity of the peripheral tissues, preserving glucose tolerance of HEI. Higher insulin levels were
44 needed in HEI than CEI cows to elicit a similar decrement of non-esterified fatty acid (NEFA)
45 concentration in prepartal IVGTT, suggesting reduced inhibition of lipolysis by insulin in HEI
46 before parturition. In conclusion, high energy intake of moderately digestible GS with low
47 concentrate feeding in close-up dry period did not have adverse effects on metabolic adaptation,
48 insulin sensitivity and body mobilization after parturition. Instead, this feeding regime was more
49 beneficial to early lactation performance than GS-based TMR diluted with wheat straw.

50 **Key words:** insulin resistance, transition dairy cow, energy intake, grass silage

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INTRODUCTION

53 In loose housing systems, group fed dairy cows are prone to extra weight gain after drying-off. Dry
54 cows easily overconsume energy relative to requirements, even when given moderate-energy maize-
55 silage (**MS**)-based diets (Janovick and Drackley, 2010). Although overfeeding may induce only
56 minor visible changes in **BCS**, overfed cows share common metabolic features with the obese cow
57 (Drackley et al., 2014). These include e.g. accretion of visceral fat depots, which drain directly to
58 the liver predisposing cows to health problems (Roche et al., 2013; Drackley et al., 2014) and
59 decreased **DMI** in early lactation (Agenäs et al., 2003; Dann et al., 2006; Janovick and Drackley,
60 2010).

61

62 The daily energy intake of dry cows offered feed ad libitum can be controlled by feeding of bulky
63 forages rich in fibre, such as wheat straw (**WS**). Restricting the energy allowance by diluting **MS**-
64 based diets with **WS** during the dry period improved **DMI** and energy balance (**EB**), and decreased
65 hepatic lipid content, and adipose tissue (**AT**) mobilization in very early lactation (Janovick and
66 Drackley 2010; Janovick et al., 2011; Mann et al., 2015). This suggests that restriction of energy
67 intake in the dry period improves metabolic balance in early lactation, despite higher non-esterified
68 fatty acid (**NEFA**) levels in transition period (Douglas et al., 2006; Looor et al., 2006; Roche et al.,
69 2015). However, the results have been more equivocal in dry cows on grass silage (**GS**) based
70 feeding strategies. No beneficial effects on **DMI** and lipid mobilization were reported when dilution
71 of **GS** or mixture of grass and **MS** with straw was compared with pure **GS** (Ryan et al., 2003, Butler
72 et al., 2011), or when the allowance of **GS** was restricted to meet the energy requirements (Kokkonen
73 et al., 2018).

74

75 Some recent studies have shown that prepartal energy intake with or without BCS changes may affect
76 maternal insulin resistance (**IR**) during the periparturient period (Zachut et al., 2013; De Koster et
77 al., 2015; Salin et al., 2017). The hyperbolic relationship between insulin secretion and insulin
78 sensitivity suggests that any environmental change in insulin sensitivity, for instance in response to
79 obesity, will be compensated by an increase in insulin secretion in response to glucose (Bergman,
80 1989; Kahn et al., 1993). This hyperbolic relationship in insulin dynamics, well established in human
81 studies, was also recently verified in transition dairy cows (Salin et al., 2017). As a result of prepartal
82 overfeeding, the AT may be more refractory to the actions of insulin (Salin et al., 2017), and cows
83 prone to lose great amounts of BW in transition period may be more resistant to insulin's effect on
84 lipolysis inhibition than cows with moderate BW loss (Zachut et al., 2013). However, some more
85 recent studies found minor effects of increased body fatness and high energy intake on inhibition of
86 lipolysis by insulin, and insulin signaling in AT of transition dairy cows (De Koster et al., 2016a;
87 Mann et al., 2016, Jaakson et al., 2018). Instead, over-conditioned cows were more insulin resistant
88 in regard to glucose metabolism and glucose transport into AT in late pregnancy than leaner cows
89 (De Koster et al., 2015; Jaakson et al., 2018).

90

91 In our former experiment (Salin et al., 2017; Kokkonen et al., 2018), we showed that high energy
92 intake of GS induced a more refractory NEFA response during the prepartal IVGTT when compared
93 with cows fed with controlled amount of GS to meet ME requirements, whereas a more pronounced
94 NEFA response was observed in overfed cows after parturition. However, a relatively short dry
95 period of 6 wk, and a feeding practice where energy oversupply was gradually restricted during the
96 close-up dry period might have contributed to the absence of differences in accretion and
97 mobilization of body reserves between high and controlled energy diets. This may have dampened
98 the metabolic and hormonal effects in the former study. Accordingly, we did not observe effects on
99 glucose and insulin responses during the transition period.

100

101 The main objective of this experiment was to determine whether increased dietary energy level by
102 ad libitum allowance of GS during an 8-wk dry period, and subsequent putative increase of body
103 condition during 8 wk dry period deteriorates pregnancy induced IR followed by abnormal metabolic
104 regulation during the transition period. Our hypothesis was that high dietary energy intake
105 accelerates accretion and mobilization of body reserves, affects hormonal control of metabolism,
106 decelerates the increase of DMI in early lactation, and thus affects production responses during the
107 first 8 wk of lactation. We also assumed that limiting of DMI by increasing the NDF-content of GS-
108 based diet with adding of WS to TMR would prevent aforementioned negative effects.

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MATERIALS AND METHODS

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Cows, Diets, and Experimental Design

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The experimental procedures were conducted under the protocols approved by the National Animal Ethics Committee in Finland (Hämeenlinna). The study was conducted with 16 Finnish multiparous Ayrshire cows, using a randomized complete block design. Experimental animals were dried off either before ($n = 12$; average 12.6 d; median 6.0 d) or on the day ($n = 4$) of the initiation of the experimental period. Those that were dried-off in advance were fed refusals of GS until 8 wk prior to expected parturition. The cows were paired according to expected calving date, parity (second through sixth), BW (733 ± 115.1 kg; mean \pm SD), and BCS (3.5 ± 0.54 ; mean \pm SD). The 305-d milk yield from the previous lactation of the cows was $10,280 \pm 1,469$ kg (mean \pm SD). Within pairs, cows were allocated to 1 of the 2 prepartal dietary treatments 8 wk before the expected parturition (57 ± 5 d before the actual calving day; mean \pm SD). The dietary treatments were (1) ad libitum access to total mixed ration (TMR) containing a mixture of 1st cut wilted GS (55%, digestible OM = 667 g/kg of DM), WS (40%, digestible OM = 457 g/kg of DM), and rapeseed meal (5%, digestible

124 OM = 700 g/kg of DM) contributing to an average of 587 g/kg of digestible OM and a ME content
125 of 9.1 MJ ME/kg of DM (controlled energy intake, **CEI**) and (2) ad libitum access to 2nd cut wilted
126 GS (high energy intake, **HEI**), containing 638 g/kg of DM of digestible OM and a ME content of
127 10.1 MJ. The silage was ensiled in round bales. Prior to baling a formic acid-based additive (AIV2
128 Plus, Kemira Ltd, Helsinki, Finland) was applied at a rate 7.4 L/tonne. The preparation of TMR is
129 described in detail in Selim et al. (2015). Grass silage or TMR was offered to cows 3 times daily at
130 0700, 1300, and 2000 h. The composition of feeds is presented in Table 1.

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132 Both diets were supplemented with 1 kg/d of commercial concentrate (Pro-Maituri 20, Raisioagro
133 Ltd., Raisio, Finland) during d 10 to d 6 prior to the expected calving date and with 2 kg/d thereafter
134 until the day of calving. The chemical composition of the concentrates is shown in Table 1. The
135 concentrate ration was fed twice daily (at 0615, and 1700 h). A commercial mineral and vitamin
136 supplement (Tunnu-Melli, Raisioagro Ltd., Raisio, Finland) was top-dressed (0.2 kg/d) once daily
137 on forage during the dry period (Supplemental Table S1). After calving, all cows were offered wilted
138 GS (average D-value 687 g/kg DM) ad libitum. The silage was cut and mixed in a stationary mixer,
139 distributed 4 times per day (at 0500, 1100, 1500, and 2000 h) by a rail-suspended distribution wagon
140 (Pellon Group, Ylihärmä, Finland). The cows were fed an increasing amount of the same commercial
141 concentrate as before calving, and a protein and mineral plus vitamin supplement (Amino-Maituri
142 30; Pihatto-Melli, Raisioagro Ltd., Raisio, Finland; Supplemental table S1). The rate of increase in
143 daily concentrate ration was similar for all cows (Supplemental table S2). The volume of concentrate
144 was 8 kg/d by the end of lactation week 1 (7 kg/d of cereal concentrate + 1 kg/d of protein
145 supplement), the maximum amount of 16 kg/d (12.5 kg/d of cereal concentrate + 3.5 kg/d of protein
146 supplement) was achieved by lactation d 32. During the first week of lactation, the daily concentrate
147 ration was fed 4 times per day (at 0615, 1000, 1645 and 1930 h) to separate feeding troughs.

148

149 The experimental animals were housed in tie stalls with saw dust bedding and rubber mats
150 throughout the dry period. The cows had continuous access to automatic water troughs and salt licks.
151 Approximately 1 wk before the expected calving, the cows were moved to individual calving pens.
152 The cows were returned to the tie stalls 2 to 5 d after parturition. The tie stalls and the calving pens
153 were equipped with forage intake control feeding stations (Insentec BV, Marknesse, the
154 Netherlands), which were fitted with separate concentrate troughs. The cows were kept in tie stalls
155 until d 10 of lactation and moved to a free-stall barn equipped with Roughage Intake Control (RIC)
156 system (Insentec BV, Marknesse, the Netherlands) with separate automatic concentrate feeders and
157 (Lely Cosmix, Lely Industries N.V., Maassluis, the Netherlands).

158

159 ***Feed and Milk Samples, Chemical Analysis, and Measurements***

160 Feed offered, and feed refused (silage and concentrates) were recorded daily. The feeds were
161 sampled weekly, and the cereal concentrate and silage samples were pooled to form a monthly
162 sample. Samples of concentrates were pooled to form a 2-mo sample. Feed samples were analyzed
163 as described by Salin et al. (2012), and dry matter content of silages was corrected for the loss of
164 volatiles according to Huida et al. (1986). VFA of the silage was determined by liquid
165 chromatographic analysis using Waters Acquity UPLC chromatography apparatus (Waters, Milford,
166 MA, USA), as described in detail by Puhakka et al. (2016). Total fat of concentrates was analyzed
167 with ether extraction and hydrolysis with HCl (SoxCap 2047 Hydrolysis Unit; FOSS Soxtec 8000,
168 FOSS Analytical, Hilleroed, Denmark). During the first week of lactation, the cows were milked
169 twice daily at 0630 and 1700 h, and thereafter until lactation week 8, the cows were milked with
170 automated milking system (Lely Astronaut A3, Lely Industries N.V., Maassluis, the Netherlands).
171 The milk yield was recorded for every milking. The milk samples were collected on 4 consecutive
172 milkings at 1, 2, 4, 6 and 8 wk after parturition, mixed with preservative (Bronopol, Valio Ltd,

173 Helsinki, Finland), and sent to a commercial laboratory (Valio Ltd., Seinäjoki, Finland) for analysis
174 of fat, protein, lactose, and urea (MilkoScan 133B analyzer; Foss Electric A/S, Hillerød, Denmark).

175

176 The cows were weighed on 2 consecutive days at 8, 6, 4, 2 and 1 wk before the expected calving
177 day, on d 1 and d 2 postpartum, and at 1, 2, 4, 6 and 8 wk after calving. The cows were always
178 weighed at the same time of day, starting at 1300 h, to minimize the influence of milking and
179 feeding. In the case of postdate pregnancies, additional weighing was done on alternate days until
180 the due date. Body condition score (Edmonson et al., 1989) was recorded by the same person
181 throughout the experiment at the same time points as weighing. The cross section of the longissimus
182 dorsi muscle (pars lumbalis) and the subcutaneous fat thickness were measured on the right
183 transversal process of the third lumbar vertebra, 2 to 3 cm medially from the lateral end at 14 days
184 prior to, and at 1, 7 and 28 d after parturition using an Aloka SSD-500 (Aloka, Tokyo, Japan)
185 ultrasound scanner with a 5.0 MHz transducer after shaving of the positions.

186

187 *Blood Samples and Intravenous Glucose Tolerance Tests*

188 Blood sampling was performed by puncture of the coccygeal blood vessels at 56, 42, 28, 21, 16, 12,
189 7, 5, 3 and 1 d before the expected calving date and at 1, 3, 5, 7, 14, 21, 28, 42 and 56 d after calving.
190 The samples were collected into evacuated collection tubes (Vacutainer; BD Medical, New Jersey,
191 USA) containing potassium ethylene diamine tetra-acetic acid (EDTA) and placed on ice. Blood
192 samples were centrifuged at 2,220 x g for 10 min to separate plasma, which was then stored at -20
193 °C for analyses of glucose, insulin, glucagon, β -hydroxybutyrate (**BHB**), NEFA, and glycerol.
194 Plasma samples for 3-methylhistidine (3-MH) were collected at 12 days prior to the expected
195 calving, and at 1, 7 and 28 d after parturition and handled as described above. The samples were
196 precipitated with 10% sulfosalicylic acid and analyzed by UPLC (Acquity UPLC, Waters, USA)

197 equipped with BEH C18 column (100mm × 2.1mm) according to manufacturer's instructions.
198 Plasma glycerol was analyzed with a direct colorimetric method (Foster et al., 1978) using a
199 commercially available kit GY105 (Randox Laboratories Limited, Crumlin, United Kingdom).
200 Plasma glucose, NEFA and insulin were analyzed as described by Salin et al. (2012) and BHB, 3-
201 MH and glucagon as described by Kokkonen et al. (2018). Intra-assay and interassay CVs for plasma
202 metabolites and hormones, liver TG and total lipids, hepatic, and AT gene expression of enzymes
203 involved in the gluconeogenesis, and fatty acid metabolism were reported by Selim et al. (2015).

204

205 Intravenous glucose tolerance tests (IVGTT) were performed 13 ± 5 d prior to the actual delivery
206 date and 9 ± 1 d postpartum at 0900 h, as described in detail by Salin et al. (2012). Briefly, on the
207 previous day sterile indwelling catheters [left: Mila 14G (Mila International, Kentucky, USA); right:
208 homemade catheter made of silicone tubing] were inserted into jugular veins and sutured to the skin
209 (Vetafil Bengen, Hannover, Germany), and a 25-cm polyvinyl chloride elongation tube (Connecta,
210 BD Medical, Franklin Lakes, NJ) was connected to the catheters. Left catheter was used for glucose
211 infusion and right one for collection of blood samples. Infusion of 0.25 g of glucose/kg of BW
212 (Glucos. 300 mg/mL, B.Braun Melsungen AG, Germany) was performed over 4.5 ± 1.4 min, and 4.6
213 ± 2.3 min (mean \pm SD) pre- and postpartum, respectively with an average infusion rate of 151 ± 39
214 mL of glucose solution/min. Blood samples were collected via catheters at -10, -5, 0, 1, 2, 3, 4, 5, 6,
215 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210 and 240 min
216 relative to the initiation of glucose infusion. The samples were handled as described above. Feed but
217 not water was withheld 2 hours before and during the IVGTT.

218

219 *Calculations and Statistical Analyses*

220 One cow from CEI was excluded from the trial 4 wk after parturition due to presumed abomasal
221 problems. The data for one cow in HEI was omitted from the statistical calculations for the first 2

222 wk of the dry period due to error in treatment allocation. Similarly, the data of one cow in CEI
223 treatment displayed abnormal NEFA response to glucose bolus prepartum, hence the data set of the
224 particular animal was considered outlier and excluded from the analysis of NEFA dynamics during
225 the IVGTT at 13 d prior to parturition.

226

227 The metabolizable energy concentration of the forages were calculated based on the digestible OM
228 concentration in DM (D-value) and the ME concentration of concentrates based on the feed tables
229 (Luke, 2017). The energy balance (EB) was calculated as the difference between the ME intake and
230 the ME requirements (Luke, 2017). A correction equation for associative effects of feeds and feeding
231 level was used when ME intake was calculated (Luke, 2017). The ECM was calculated according
232 to Sjaunja et al. (1991).

233

234 The net incremental area under the response curve (AUC) for plasma glucose, insulin and NEFA
235 during the first 60 and 240 min of IVGTT was calculated as described by Salin et al. (2017). The
236 decrement of NEFA was calculated by subtracting the nadir concentration from the basal NEFA
237 concentration. Clearance rate (CR; %/min) of metabolites and insulin were calculated using PROC
238 NLIN of SAS (version 9.3) as described previously (Pires et al., 2007; Salin et al., 2012). For each
239 IVGTT, the Minimal Model (MM; Bergman, 1989) was applied to glucose and insulin curves using
240 a commercial software (MinMod Millennium, MINMOD Inc., Pasadena, CA) with methods described
241 previously (Boston et al., 2003; Salin et al., 2012). Briefly, the model provides values for insulin
242 sensitivity (SI; $\times 10^{-4} \text{ min}^{-1}/\mu\text{IU/mL}$), defined as the fractional rate of glucose uptake per unit of
243 plasma insulin; glucose effectiveness (Sg; min^{-1}), characterizes non-insulin mediated glucose
244 disposal; acute insulin response to glucose (AIRG; $\mu\text{IU/mL/min}$), representing the endogenous
245 insulin response to a glucose bolus; and disposition index (DI), a measure of overall glucose
246 tolerance, and insulin responsiveness corrected for changes in SI. DI is calculated as the product of

247 SI and AIR_G. For the evaluation of the NEFA dynamics during the IVGTT, a NEFA model was used
248 (Boston and Moate, 2008).

249

250 Data for feed intake, milk production and blood hormone and metabolite concentrations, BW, BCS,
251 EB, and postpartal plasma 3-MH and back muscle diameter were analyzed as repeated measures
252 ANOVA using the MIXED procedure of SAS version 9.3 (SAS Institute, Cary, NC, USA).
253 Prepartum and postpartum data were analyzed separately. Measurements of DMI, EB, and milk
254 production were reduced to weekly means before statistical analysis. Three covariance structures
255 were tested for each variable analyzed; compound symmetry (CS), unstructured (UN) and
256 autoregressive order 1 [AR(1)]. For unequally spaced measures, spatial power [SP(POW)] was used
257 instead of AR(1). The statistical model included fixed effects of treatment, time (day or week relative
258 to calving), the interaction between treatment and time (diet x time), and a random effect of block
259 and interaction between block and time. Degrees of freedom were estimated by using the Kenward-
260 Roger option in the model statement. The covariance structure that resulted in the smallest
261 Schwarz's Bayesian information criterion was used.

262

263 The data of BW, BCS and EB at particular time points and their changes, prepartal plasma 3-MH and
264 back muscle diameter, as well as data derived from the IVGTT were analyzed by ANOVA with a
265 model including fixed effect of treatment and random effect of block using the PROC MIXED of
266 SAS (version 9.3; SAS Institute Inc., Cary, NC). A fixed effect of interval between the sampling day
267 and the actual day of parturition was included in the statistical model of the prepartal IVGTT data.

268

269 Prior to statistical analysis, residuals of each variable were checked for normality using the MIXED
270 and UNIVARIATE procedures of SAS. To correct for the deviations from normality and
271 homoscedasticity of the residuals for the data of each variable, the variables were subjected to

272 reciprocal or log-transformation (LN), when needed. The Friedman's nonparametric test was used
273 whenever the above mentioned normalization processes were not effective, as was the case in the
274 MM derived prepartal estimates SI and Sg. All values in the tables and figures are reported as least
275 squares means and standard error of the mean (SEM). Back-transformed least squares means, and
276 SEM are reported from the analysis of transformed data. The relationships between plasma
277 concentrations and calculated parameters describing insulin sensitivity during IVGTT were
278 investigated by Spearman's correlation analysis, using the CORR procedure of SAS. The effects were
279 considered statistically significant at $P < 0.05$, and trends for effects are discussed at $0.05 \leq P < 0.10$.

280

281

RESULTS

282

Diets and Intakes

284 The analyzed composition of prepartum and postpartum diets is shown in Table 1. The formulation
285 of TMR was successful and the targeted difference in the NDF-content of the diets between HEI and
286 CEI was achieved. The average NDF-content of the GS of HEI was lower than the NDF levels
287 measured from the preliminary samples, leading to an average NDF difference of 120 g/kg of DM
288 between the treatments. The inclusion of rapeseed meal in the TMR was successful in balancing the
289 CP content of CEI close to the CP content of GS fed in HEI. The digestible OM content (D-value) of
290 the CEI was, as expected, lower than that of HEI.

291

292 The experimental design led to expected significant differences in DMI and intakes of other nutrients
293 as well as in ME-intakes between the treatments during the prepartum period (Table 2). The total DM
294 and forage DM intake (Figure 1) of HEI was 2.2 and 2.9 kg/d higher in HEI than in CEI during the
295 dry period, respectively ($P < 0.01$). The DMI expressed as % of BW tended to be greater during the
296 entire dry period in HEI than in CEI ($P = 0.05$) and the significant diet x time interaction showed that

297 the difference in DMI was largest on wk -8 and -6 relative to parturition, averaging 2.0% and 1.5%
298 of BW (SEM 0.12; P = 0.04). The EB during the dry period (Figure 2) was approximately 30 %
299 greater in HEI than in CEI (P < 0.001) and the average energy intake was 141% and 108 % (SEM
300 5.46; P < 0.001) of the requirements (Luke, 2017) in HEI and in CEI, respectively. After parturition,
301 the dry period feeding had no effect on total DMI and intakes of nutrients except for a significant
302 difference in concentrate DMI. The HEI cows had higher intake of concentrates than CEI cows from
303 wk 4 onwards (diet x time P = 0.05), resulting in 0.4 kg/d greater postpartal average DMI of
304 concentrates in HEI than in CEI (SEM 0.09; P = 0.003). The energy balance was not affected by the
305 prepartal dietary treatment after the parturition (P = 0.46).

306

307 ***Body Reserves***

308 The cows were dry for an average of 67 ± 11.1 d (mean \pm SD). The parameters describing the changes
309 of body reserves are shown in Table 3. The initial BW and BCS were not different between
310 treatments, by design. However, the significant diet x time interactions in BW (P = 0.005) and BCS
311 (P = 0.02) during the dry period indicated steeper increment of BW and BCS in HEI than in CEI
312 (Supplemental figure S1 and S2). The BW change from d -56 to d -5 prior to parturition was greater
313 in HEI than in CEI (P = 0.03) and accounted for a total increment of 75.0 vs. 40.8 kg (SEM 9.1),
314 respectively. The BCS change tended to be greater in HEI than in CEI prepartum and increased in
315 total 0.37 vs. 0.14 (SEM 0.08; P = 0.07) from d -56 to d -5. The birth weights of calves did not differ
316 between the treatments. We found no treatment and no diet x time interaction effects on either BW,
317 BCS or changes in these parameters during the early lactation. Back muscle diameter on -14 d
318 prepartum was similar in both groups but declined to a smaller average value in CEI than in HEI
319 during the first 4 wk of lactation (P = 0.01). We observed no differences in back fat thickness.

320

321 ***Plasma Metabolites and Hormone Concentrations***

322 Basal plasma hormone and metabolite concentrations are depicted in Table 4. The concentration of
323 plasma glucose before parturition was higher in HEI than in CEI, and the difference was most evident
324 during the last 2 wk of pregnancy (diet x time, $P = 0.06$; Figure 3). After parturition, the HEI cows
325 tended to have higher glucose concentrations than CEI cows ($P = 0.09$). We found a tendency for
326 greater insulin levels in HEI than in CEI during the dry period ($P = 0.07$), the difference being most
327 pronounced during the last 4 wk prior to parturition (diet x time, $P = 0.08$; Figure 4). The insulin
328 concentration did not differ between treatments after parturition. We did not observe a difference in
329 either the plasma glucagon concentration or in the glucagon to insulin ratio during the experimental
330 period. The average plasma concentration of NEFA did not differ prepartum, but the rise of NEFA
331 concentration during the last week of pregnancy tended to be steeper in CEI than in HEI (diet x time,
332 $P = 0.07$; Figure 5). After parturition, there were no differences in the average plasma NEFA
333 concentration. Plasma BHB concentrations were greater in HEI than in CEI during the dry period (P
334 $= 0.006$; Figure 6). After calving, the BHB levels increased until 2 wk and 4 wk of lactation in HEI
335 and CEI, respectively. CEI cows had higher BHB at wk 6 and 8 (diet x time, $P = 0.07$). Plasma
336 glycerol concentrations were not affected by the prepartal dietary treatments. Plasma 3-MH tended
337 to be higher in CEI than in HEI on -12 d prepartum ($P = 0.09$), while no differences in plasma 3-MH
338 were found after parturition.

339

340 ***Milk Production and Composition***

341 The milk production parameters are depicted in Table 5. The average milk yield and the ECM were
342 not affected by the treatments. However, a significant diet x time interaction was observed, indicating
343 higher milk yield in HEI than CEI from week 5 onwards, the average difference being 5.3 ± 1.6 kg
344 on wk 7 and 8 ($P = 0.007$; Supplemental figure S3). The feed efficiency (ECM/kg DMI) was not
345 affected by prepartal diet. Increase in feed efficiency tended to be greater in HEI than in CEI during
346 the first 2 wk of lactation, the difference was most pronounced in wk 2 (diet x time, $P = 0.07$). Milk

347 fat-% was greater in HEI vs. CEI on lactation wk 1, 2, and 6 (diet x time, $P = 0.003$). Milk protein
348 and lactose concentrations were not different between treatments. Milk urea tended to be higher in
349 HEI than in CEI over time, the difference being most pronounced on lactation wk 4 (diet x time, $P =$
350 0.09). The fat yield was not different between treatments. The protein yield tended to be higher in
351 HEI vs. CEI from wk 6 onwards, the difference being greatest on wk 8 (diet x time, $P = 0.05$). The
352 lactose yield was higher in HEI than in CEI on wk 8 (diet x time, $P = 0.01$).

353

354 ***Glucose, Insulin, and NEFA Dynamics During the IVGTT***

355 Basal and peak concentrations, and CR of glucose prepartum were not affected by the dietary
356 treatments (Table 6). However, glucose area under the response curve was smaller in HEI than in
357 CEI during the prepartal IVGTT (AUC_{240} ; $P = 0.02$; Figure 7A). Prepartal diet affected the
358 endogenous insulin response to glucose infusion during the IVGTT at 13 d prior to parturition (Figure
359 7B). The peak concentration ($P = 0.01$) and the AUC of insulin during the first hour of the IVGTT (P
360 $= 0.03$) were greater in HEI than in CEI, and the AUC during the 240 min IVGTT tended to be greater
361 in HEI than in CEI ($P = 0.05$). The effect of the interval between the IVGTT and the day of calving
362 was significant during the prepartal IVGTT for insulin peak concentration and insulin AUC ($P <$
363 0.05), indicating that cows closer to their due date had smaller insulin AUC and lower peak
364 concentrations. The basal and nadir concentrations of NEFA, as well as CR and AUC did not differ
365 between treatments pre- and postpartum (Figure 7C). However, during the prepartal IVGTT the
366 NEFA Model derived parameter for latency was greater ($P = 0.04$) and FFA0 tended to be smaller (P
367 $= 0.09$) in HEI than in CEI.

368

369 The dry period dietary treatment had minor effect on MM estimates, as the only significant difference
370 was higher AIRg in HEI than in CEI ($P = 0.002$) during the prepartal IVGTT (Table 6). The effect
371 of the interval between the IVGTT and the day of calving was significant during the prepartal IVGTT

372 for DI ($P = 0.03$), showing that the closer the due date the lower the DI. The values of Sg, SI and DI
373 did not differ between the treatments at either time points ($P > 0.10$).

374

375 We found no treatment differences in glucose, insulin and NEFA responses to infusion of glucose
376 after parturition ($P > 0.10$; Figure 8). The basal NEFA had a strong positive correlation with NEFA
377 decrement both in prepartal ($r = 0.98$; $P < 0.001$) and postpartal ($r = 0.74$; $P < 0.001$) IVGTT.
378 Similarly, basal NEFA had a negative association with NEFA AUC₆₀ both in prepartal ($r = -0.69$; P
379 < 0.004) and in postpartal IVGTT ($r = -0.50$; $P = 0.049$).

380

381

DISCUSSION

382 The main objective of this study was to investigate whether controlling energy intake of dairy cows
383 by diluting a moderately digestible GS with WS during the 8 wk dry period affects whole body IR,
384 and tissue deposition and mobilization during the early lactation in comparison to ad libitum access
385 of GS. We assessed these conditions by measuring the changes of plasma metabolite and hormone
386 concentrations under basal and stimulated conditions, as well as by investigating the changes on
387 BCS and BW, and on production performance of early lactation. Given that GS is the main source
388 of forage for dairy cows in many Northern European countries during the indoor feeding period, we
389 have previously assessed the effects of different allowances of sole GS during the dry period on
390 metabolic and production responses (Salin et al. 2017; Kokkonen et al., 2018). In the present
391 experiment, GS as a sole forage source, and GS diluted with straw fed ad libitum were chosen for
392 comparison to investigate a more practical feeding regime for loose housing systems.

393

394 *Dry matter intake*

395 The prepartal DMI was well maintained in CEI cows in accordance to earlier studies showing that
396 controlling of energy intake either by restriction of the amount of TMR or GS (Agenäs et al., 2003;
397 Kokkonen et al., 2018) or the composition of the diet (Rabelo et al., 2003; Vickers et al., 2013)
398 prevented the decline in DMI during the last weeks of pregnancy. The moderate decline in prepartal
399 DMI of HEI cows during the last 4 wk prepartum in the current study (0.9 ± 0.73 kg/d) is in
400 agreement with Agenäs et al., (2003) who reported an average of 1 kg/d decline in DMI during the
401 same time period in overfed cows on GS-based diets. In comparison, the DMI decline in cows fed
402 ad libitum GS with or without barley straw in a series of experiments during the final 5 wk of the dry
403 period (NDF range 452 to 689 g/kg DM) was in average 1.47 kg/day, half of which occurred during
404 the week preceding calving (Dewhurst et al., 2010). Although we did not investigate digestibility of
405 the nutrients in this study, the lower intake of CEI is at least partly attributable to the lower nutrient
406 digestibility resulting from inherent characteristics of straw fiber (Dewhurst et al., 2000).

407

408 We did not find any difference in postpartal DMI in agreement with other studies with moderate (30
409 to 40 %) overfeeding of energy during the dry period (Butler et al., 2011; Mann et al., 2015;
410 Kokkonen et al., 2018). These results contrast with previous studies showing that high energy diets
411 (150 to 160% of energy requirement) during late pregnancy resulted in lower feed intake during the
412 early lactation in MS-based diets (Dann et al., 2006; Douglas et al., 2006; Janovick and Drackley,
413 2010). The moderately, but statistically significantly lower concentrate DMI in CEI than HEI after
414 parturition, especially during the second month of lactation, could possibly be a carry-over effect of
415 the prepartal TMR. The inclusion of WS into diet of CEI may have affected rumen adaptation
416 mechanism, e.g. papillae development and structural changes (Odongo et al., 2006; Steele et al.,
417 2011). The very moderate amount of concentrates given for only 12 ± 5 d (mean \pm SD) prepartum
418 may have resulted in a too low non-structural carbohydrate (NSC) intake of CEI in order to optimize
419 the adaptation to a concentrate rich lactation ration after parturition. In fact, higher contents of

420 digestible carbohydrate, such as starch, enhanced the overall VFA absorption capacity via rumen
421 papillae development (Goodlad, 1981; Dirksen et al., 1985).

422

423 *Plasma Metabolites and Hormones*

424 Higher prepartal concentration of basal glucose in cows fed high energy diets compared with
425 controlled energy diets during the entire 8 wk dry period is analogous with earlier research (Douglas
426 et al., 2006; Janovick et al., 2011; Mann et al., 2016), whereas we did not observe a corresponding
427 difference when different amounts of GS were fed to dry cows during the 6 wk dry period
428 (Kokkonen et al., 2018). The higher blood glucose concentration in HEI is most likely a result of
429 increased availability of propionate for hepatic gluconeogenesis (Janovick et al., 2011; Mann et al.,
430 2016) as the TMR with high amounts of straw lacks precursors for gluconeogenesis. Correspondent
431 with higher glucose levels, the HEI cows tended to have higher insulin concentration during the
432 prepartal period, analogous to other recent studies showing that energy overfeeding during the dry
433 period resulted in higher prepartum insulin concentrations compared with cows fed restricted energy
434 (Holtenius et al., 2003; Douglas et al., 2006; Janovick et al., 2011). The higher, but still moderate
435 plasma BHB in HEI than in CEI cows prepartum is most likely a consequence of a greater ruminal
436 butyrate production due to higher intake of GS, and not an indicator of metabolic imbalance (Roche
437 et al., 2013). In line with this, the mRNA expression of mitochondrial CPT1 activity in the liver at
438 d -14, 1 and 7 d relative to parturition was not changed in the current animals (Selim et al., 2015)
439 suggesting that the translocation of FA derivatives to hepatic mitochondrial beta-oxidation was
440 unchanged around parturition. The results agree with earlier studies reporting lower prepartal BHB
441 in cows on controlled energy diets than in cows on higher energy diets with a normal or low BCS at
442 dry-off (Mann et al., 2015; Little et al., 2016). Butler et al., (2011) showed greater blood
443 concentrations of BHB in GS fed cows than in cows fed a wheat-based TMR at 1 wk before

444 parturition and during the first 4 weeks postpartum without any treatment effect on incidence of
445 ketosis.

446

447 The tendency for higher plasma glucose in HEI after parturition is inconsistent with the absence of
448 differences in plasma glucagon concentration or glucagon to insulin ration suggesting no differences
449 in gluconeogenesis from amino acids and lactate (Aschenbach et al. 2010). The higher plasma
450 glucose is also inconsistent with the results of Selim et al. (2015) from the same cows suggesting
451 attenuated increase of hepatic gluconeogenic activity from propionate in HEI, as the hepatic PCK1
452 mRNA expression was downregulated in HEI but not in CEI at d 1 and d 7 of lactation when
453 compared with d -8. The higher BHB concentration in CEI than HEI at d 42 and 56 indicates
454 compensation of insufficient supply of glucose precursors, as suggested by lower concentrate DMI.
455 The higher BHB concentration may have also served as tissue protective mechanism in CEI, because
456 BHB has been shown to inhibit basal AT lipolysis in vitro in a dose-dependent matter (Van der Drift
457 et al., 2013). Thus, the feed-back mechanisms may have prevented toxic effects of high BHB levels
458 on tissues by shutting down further release of additional NEFA (Rukkwamsuk et al., 1998). This
459 may explain the lack of differences in NEFA concentrations between CEI and HEI at d 42 and 56.

460

461 ***Indicators of Tissue Accretion and Mobilization***

462 The cows in HEI gained more BW during the 8 wk dry period. Although the difference in BW gain
463 between energy levels was greater in the present experiment than in our previous experiment
464 (Kokkonen et al. 2018), we expected a larger response of surplus EI on BW and BCS gain before
465 calving. According to the Finnish nutrient requirements (Luke 2017) an average ME-difference of
466 34 MJ/d between the treatments during the dry period should have resulted in an approximate
467 difference of 1 kg/d in daily BW change instead of reported value of 0.6 kg/d. Thus, our findings

468 support recent studies postulating that ME requirements of pregnant cows may be underestimated in
469 different energy calculation systems, and that the actual requirements for maintenance and lactation
470 may be larger than reported (Mandok et al., 2013; Kokkonen and Vanhatalo, 2014). Moreover, it
471 seems that cows in a fairly good body condition at dry-off, as were all the cows in our study, do not
472 gain a lot of BCS (0.3 units in average) in GS-based feeding during an 8 wk dry period in agreement
473 with our recent study with a shorter dry period of 6 wk (Kokkonen et al., 2018). However, higher
474 prepartal energy intake affected the onset of excessive tissue mobilization near calving. The tendency
475 for a more pronounced rise of plasma NEFA and higher plasma 3-MH in CEI in the weeks preceding
476 calving indicates that CEI cows initiated the mobilization process at an earlier stage precalving.

477

478 In contrast to studies showing that postpartal BW losses and NEFA concentrations were lower when
479 dietary energy intake was restricted prepartum (reflecting positive effect of dry period feeding level
480 on energy balance postpartum) in TMR fed dairy cows (Douglas et al., 2006; Janovick and Drackley
481 2010; Janovick et al., 2011), we did not find any differences neither in BW and BCS losses nor in
482 plasma NEFA concentrations after parturition. Our data suggest that cows on GS-based diets pre-
483 and postpartum are not prone to large changes in BCS and BW after parturition, concordant with
484 studies on similar forage type (Agenäs et al., 2003; Vickers et al., 2013; Little et al., 2016; Kokkonen
485 et al., 2018). More pronounced differences in prepartal BW changes on GS or MS diets have resulted
486 in increased AT mobilization after parturition (Kokkonen et al., 2005; Douglas et al., 2006). Our
487 results suggest that overfeeding on GS-based diets has only moderate effect on metabolic flexibility
488 of transition dairy cows. Conversely, a recent review underlined negative effects of over-
489 conditioning on MS-based diets with excessive AT mobilization on metabolic disorders of cows in
490 early lactation (Drackley and Cardoso, 2014). The observed strong positive correlation of initial BCS
491 and that of the BCS at 5 d prior to parturition is in agreement with Kokkonen et al. (2018). These
492 findings support the earlier suggestions (Friggens et al., 2004) implying that dairy cows have an

493 inherent level of body reserves towards which their metabolism is aiming for during the late lactation
494 and in the transition period in order to restore the genetically predestined body condition. Further,
495 the absence of differences in BCS at calving or in the mobilization of body tissues after parturition
496 imply that neither the BCS at calving (as suggested by e.g. Roche et al., 2009; 2015; Pires et al.,
497 2013) nor the prepartal energy intake per se were determinants of the postpartal performance after
498 parturition in the current study.

499

500 ***Production Responses***

501 The milk yield was moderately affected by prepartal feeding, as we observed greater milk yield in
502 HEI than CEI from wk 4 onwards. Earlier studies comparing GS and mixture of GS and straw
503 prepartum reported increased milk yield with GS (Dewhurst et al., 2000; McNamara et al., 2003;
504 Ryan et al., 2003). However, those studies showed that the greatest influence on milk production
505 performance was observed during the early weeks after calving. This probably resulted from
506 improved BCS at calving on GS, because the initial BCS at beginning of the dry period feeding was
507 below 2.75 (McNamara et al., 2003; Ryan et al., 2003). Apparently, an identical temporal effect on
508 the milk yield with the current one was found in our former experiment (Kokkonen et al., 2018)
509 conducted at the same institute with genetically similar animals from the same herd, fed with
510 different amounts of GS during the dry period. However, as opposed to current results, the cows on
511 controlled energy diet cows tended to produce more milk in the earlier study (Kokkonen et al., 2018).
512 A common element of these two studies was a slightly lower intake of concentrates during the early
513 lactation in those animals producing less milk after the first month. In the present study, it seems
514 that a large proportion of straw in the dry period diet or the short concentrate feeding period, or both,
515 had a negative effect on adaptation of rumen to postcalving diets and subsequent production
516 responses, as discussed earlier in this paper. With a moderately digestible GS, there is no need to
517 dilute the feed composition by adding of straw, as this dry period feeding practice had moderate

518 negative carry-over effects on postpartal concentrate intake and consequently on early lactation
519 performance in multiparous cows of good body condition at calving. The lack of treatment effects
520 on milk fat and protein contents is in line with earlier experiments on GS-based diets (Agenäs et al.,
521 2003; Kokkonen et al., 2018) reflecting the absence of any obvious differences in energy balance
522 and lipid mobilization during the early lactation. This also indicates that early lactating cows have
523 a potential to compensate for changes in prepartal nutrient intake providing that the cows receive a
524 standard lactation ration of high quality (Agenäs et al., 2003).

525

526 *IVGTT*

527 The observed greater insulin response of HEI cows before calving (greater AUC of insulin and AIRg)
528 and the subsequent smaller AUC of glucose suggest that glucose tolerance of HEI was preserved
529 before parturition. However, any environmental change in insulin sensitivity, for instance in response
530 to obesity, will be compensated by an increase in insulin secretion in response to glucose (Bergman,
531 1989; Kahn et al., 1993). Given the former, we may speculate that the greater insulin secretion in
532 HEI was a compensatory mechanism in response to reduced insulin sensitivity of the peripheral
533 tissues to preserve the glucose tolerance. Salin et al. (2017) found no effect of higher energy intake
534 on insulin response when comparing different GS allowances during the dry period. Similarly,
535 overfeeding of energy in the close-up dry period alone or during the entire dry period did not affect
536 insulin response during IVGTT in cows on TMR based on MS plus WS (Schoenberg et al., 2012;
537 Mann et al., 2016). In contrast, Jaakson et al., (2018) reported higher insulin response to IVGTT at -
538 21 d in cows with BCS > 3.75 (1-5 scale) when compared with thin cows (BCS < 3.0) on GS and
539 hay based TMR. However, as opposed to current results, the over-conditioned cows had larger
540 glucose AUC than the thinner cows, indicating a higher degree of IR. The discrepancies between
541 studies may stem from different timing of the challenges, and from differences in feed composition,
542 breed, and from dissimilarities in initial and achieved BCS between treatments.

543

544 In agreement with Salin et al. (2017) reporting attenuated NEFA response of overfed cows during
545 the prepartal IVGTT and enhanced sensitivity of AT after parturition, we found indications of dietary
546 effects on insulin's action on inhibition of lipolysis on GS-based diets. However, the effects in this
547 study were evident only before parturition, as the HEI cows needed greater insulin concentrations to
548 elicit a similar NEFA response than CEI cows in prepartal IVGTT. The former may reflect reduced
549 AT sensitivity to insulin in response to overfeeding. The greater latency of NEFA response during
550 the prepartal IVGTT in HEI than in CEI reinforces the suggestion that insulin sensitivity in AT of
551 HEI was affected by dry period energy intake. The latency is thought to result from the time it takes
552 for the challenge to trigger the suppression of lipolysis (Boston and Moate, 2008). The extended
553 latency period of HEI cows in prepartal IVGTT, together with the higher insulin response and a
554 similar eventual NEFA suppression, may insinuate that the antilipolytic action of insulin was
555 compromised in cows with higher BW gain during the dry period. Similarly, cows that were losing
556 high levels of BW had more refractory AT to insulin both pre and postpartum (Zachut et al., 2013).
557 By contrast, Mann et al., (2016) did not find any effect of different energy intake on NEFA response
558 during the transition period, neither did Marett et al., (2015) during different stages of lactation.
559 Insulin response of the glucose metabolism, but not that of fatty acid metabolism, was negatively
560 associated with excessive accumulation of AT in late pregnant dairy cows as assessed by HEC test,
561 while insulin sensitivity in AT of over conditioned cows with greater adipocytes was preserved in
562 vitro (De Koster et al., 2015, 2016). Recently, glucose transporter 4 protein synthesis in AT of
563 overconditioned cows was reduced, suggesting a more severe IR prepartum, while no differences in
564 insulin signaling potential were found relative to thinner cows (Jaakson et al., 2018).

565

566 We showed that basal NEFA had a strong correlation with NEFA decrement and NEFA AUC during
567 IVGTT both pre- and postpartum. Analogous results have been reported in studies where insulin

568 sensitivity was assessed by different methods (Patton et al., 2009; Schoenberg et al., 2012; De Koster
569 et al., 2015; Salin et al., 2017). The results may imply that the NEFA decrement during stimulated
570 conditions is not only a result of the direct insulin response (the insulin AUC) after a glucose bolus
571 but is also partially mediated by the secondary effect of insulin and lipolytic agents on basal lipolysis
572 prior to IVGTT. Indeed, increased fatness of dairy cows amplified the lipolytic response of AT to
573 catecholamine stimulation (Kokkonen et al., 2005) especially in the dry period (Theilgaard et al.,
574 2002). Similarly, both the basal and stimulated in vitro AT sensitivity to lipolytic agents were greater
575 in over-conditioned than in normal conditioned cows before parturition (De Koster et al., 2016a).
576 Furthermore, in humans at least, the inhibitory action of insulin on AT lipolysis is dependent on the
577 prevailing lipolytic activity, such that the antilipolytic effect of the hormone is more pronounced
578 when the rate of lipolysis is augmented, probably due to increased insulin receptor and signal
579 transduction activity (Zierath et al., 1998). Finally, the shutdown of lipolysis during the IVGTT
580 challenge of ruminants may also be partly directly regulated by glucose, which is the metabolic driver
581 of the degree of NEFA suppression in human AT cells (Arner et al., 1983; Qvisth et al., 2004).

582

583 Whilst it was not our main intention to compare insulin responsiveness and sensitivity of tissues at
584 different time points in the current study, we found that MM derived values of SI and DI for the
585 IVGTT at -13 d were numerically smaller than at +9 d. When the relationship of SI and AIRg (= DI) is visualized as shown in Figure 9, the left and upward shift of the DI values in HEI at -13 d
586 relative to parturition underpin that the compensatory insulin secretion to match the decrease in
587 insulin sensitivity was sufficient. However, as the DI reflects the ability of the β -cells of the
588 pancreatic islets to compensate for IR (Bergman, 1989) the very low prepartal values of DI point to
589 an insulin insensitive pancreas in response to glucose and to an overall lower compensation for
590 decreased insulin sensitivity in all animals indicated also by very low SI values in agreement with
591 earlier studies (Stanley, 2005; De Koster et al., 2016b; Salin et al., 2017). Further, in comparison to
592

593 postpartal values across the treatments, the transformation of the values to the right indicate that as
594 the value of SI is greater after parturition there is no need to an additional compensation in insulin
595 secretion indicated by lower DI and AIRg after parturition, in agreement with Salin et al., (2017).
596 Indications of improvement in overall sensitivity of tissues to insulin in early lactation have been
597 published based on different determination methods of IR (Stanley, 2005; Oliveira et al., 2016).
598 Opposing results suggest that (peripheral) insulin sensitivity of dairy cows is not profoundly changed
599 during the transition period, regardless of prepartal feeding and degree of body fat mobilization
600 (Mann et al., 2016; de Koster et al., 2016a; Weber et al., 2016). Varied results from range of different
601 methods of studying IR in cows in late pregnancy and early lactation clearly highlight the challenges
602 in investigation of the transition period metabolism (Marett et al., 2015; De Koster et al., 2017).
603 Additional research is needed to elicit a consensus on the applicability of the Minimal Model in
604 periparturient dairy cows.

605

606 Overall, as our previous experiment showed, that when the dry period was relatively short, and the
607 oversupply of energy on GS-based diets was gradually decreased in the close-up dry period, the
608 effects of overfeeding on whole body insulin sensitivity were only evident in the level of AT (Salin
609 et al., 2017). By contrast, GS fed ad libitum for a longer period of 8 wk, as in the present study,
610 generating a more positive EB in dry cows, did not only induce a delayed response to insulin in AT,
611 but also increased insulin secretion in response to IVGTT near parturition. This, in turn accelerated
612 plasma glucose disappearance and inhibited glucose output or both, preserving peripheral glucose
613 tolerance. Not only the difference in energy intake, but also the greater potential of GS to supply
614 glucogenic precursors in comparison to the mixture of GS and WS contributed to the observed
615 effects on glucose and NEFA dynamics orchestrated via insulin.

616

617

CONCLUSIONS

618

619 Overfeeding energy in grass silage-based diet resulted in elevated BW and BCS gain during the dry
620 period. However, given the average difference of 40% in prepartal energy intake between the
621 treatments, we observed smaller than expected differences in BW gain during the dry period. Contrary
622 to the hypothesis, high energy intake during the dry period did not affect mobilization of body
623 reserves and feed intake after calving, whereas milk yield was greater from wk 5 onwards in overfed
624 cows. Parameters from prepartal IVGTT indicated that overfed cows had more pronounced insulin
625 response to glucose load and a smaller glucose AUC, reflecting preserved glucose tolerance. Further,
626 the delayed NEFA response during prepartal IVGTT suggest attenuated inhibition of lipolysis in
627 response to oversupply of energy. The dietary differences in propionic acid availability leading to
628 lower prepartal glucose and insulin levels in TMR fed cows most likely contributed to the observed
629 responses during the IVGTT prepartum. These effects of prepartal feeding on insulin sensitivity did
630 not carry over to the early lactation. Our results suggest that controlling energy intake of dry cows by
631 dilution of moderately digestible grass silage by straw is not beneficial for optimally conditioned
632 cows. In conclusion, ad libitum feeding of moderately digestible grass silage during the dry period
633 had only transient effects on metabolic adaptation and insulin sensitivity during the transition period.
634 This feeding regime was more favorable to early lactation performance than ad libitum fed TMR of
635 grass silage diluted with WS.

636

637

ACKNOWLEDGEMENTS

638

639 The authors gratefully appreciate the assistance of Juha Suomi and the staff at the research farm of
640 the University of Helsinki for their care of experimental animals and that of the laboratory staff of
641 the Department of Agricultural Sciences, University of Helsinki. We also extend our gratitude to
642 Rashid Safari for the assistance in handling the data. This study was funded by the Finnish Ministry

643 of Agriculture and Forestry. Raisio plc Research Foundation (Raisio, Finland) and the Agricultural
644 Research Foundation of August Johannes and Aino Tiura (Espoo, Finland) supported the first author
645 financially.

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889 **Table 1.** Chemical composition and calculated energy content of forages and concentrates in dietary treatments CEI¹ and HEI²

Item	TMR ³ CEI	Grass silage ⁴ CEI	Wheat straw ⁵ CEI	Rapeseed meal ⁶ CEI	Grass silage ⁷ HEI	Grass silage ⁸ Lactation	Concentrate ⁹	Protein supplement ¹⁰
DM, g/kg	272	201	813	880	273	288	873	871
Ash, g/kg DM	60	63	74	68	84	85	69	83
CP, g/kg DM	122	146	48	368	129	146	196	288
Ether extract, g/kg DM	-	-	-	57	-	-	50	78
NDF, g/kg DM	651	595	775	273	531	527	211	202
ME, MJ/kg DM	9.1	10.7	6.4	11.4 ¹¹	10.1	11.0	12.8 ¹²	13.0 ¹²
D-value, g/kg DM ¹³	587	668	457	-	638	687	-	-
MP, g/kg DM	-	80.5	42.0	169 ¹¹	62.4	67.3	119 ¹²	140 ¹²

890 ¹CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

891 ²HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

892 ³Total mixed ration fed to CEI during the dry period

893 ⁴Grass silage in TMR fed to CEI during the dry period. Mean fermentation characteristics: pH 3.9; (% in DM) lactic acid (3.8), acetic acid (1.5),
894 butyric acid (0.034), propionic acid (0.14), sugars (6.7); (% of total N) ammonium-N (6.7); soluble N (62).

895 ⁵Wheat straw in TMR fed to CEI during the dry period.

896 ⁶Rapeseed meal in TMR fed to CEI during the dry period

897 ⁷Grass silage fed to HEI during the dry period. Mean fermentation characteristics: pH 4.1; (% in DM) lactic acid (3.3), acetic acid (0.70), butyric
898 acid (0.00), propionic acid (0.12), sugars (1.6); (% of total N) ammonium-N (5.5); soluble N (47).

899 ⁸Grass silage fed during lactation. Mean fermentation characteristics: pH 4.4; (% in DM) lactic acid (3.3), acetic acid (1.4), butyric acid (0.20),
900 propionic acid (0.12), sugars (1.1); (% of total N) ammonium-N (6.8); soluble N (61)

901 ⁹Concentrate fed during the last 12 ± 5 d (mean ± SD) of pregnancy and during lactation.

902 ¹⁰Protein supplement fed during lactation.

903 ¹¹Values adopted from Luke (2017)

904 ¹²Values provided by the manufacturer (Raisio Oy Ltd; Raisio, Finland)

905 ¹³In vitro digestible organic matter in DM

906

907 **Table 2.** Effect of dry period energy intake and diet composition on DMI and energy balance

Item	CEI ¹	HEI ²	SEM	P-value	
				Diet	Diet x Time
Prepartum					
Forage DM, kg/d	10.8	13.7	0.46	<0.001	0.74
Concentrate, DM, kg/d ³	1.3	1.4	0.10	0.77	0.03
Total DM kg/d	12.0	14.2	0.50	0.002	0.64
DMI, % of BW	1.58	1.86	0.10	0.05	0.04
NDF, kg/d	7.5	7.3	0.3	0.70	0.29
MP, kg/d	0.86	0.89	0.03	0.41	0.54
ME, MJ/d	109	144	4.8	<0.001	0.52
ME-balance, MJ/d	8.52	39.4	4.97	0.004	0.51
Postpartum					
Silage DM, kg/d	11.2	11.3	0.54	0.88	0.82
Concentrate DM, kg/d	10.9	11.3	0.09	0.003	0.05
Total DM, kg/d	22.1	22.6	0.57	0.52	0.57
NDF, kg/d	8.17	8.33	0.29	0.71	0.56
MP, kg/d	2.10	2.17	0.41	0.27	0.34
ME, MJ/d	262	270	6.56	0.42	0.57
ME balance, MJ/d	-47.3	-56.4	8.69	0.46	0.25

908 ¹CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

909 ²HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

910 ³Concentrate fed during the last 12 ± 5 d (mean ± SD) of pregnancy

911

Table 3. Effect of dry period energy intake and diet composition on body composition

Item	CEI ¹	HEI ²	SEM	P-value
Prepartum				
Body condition score (-8 wk)	3.5	3.4	0.20	0.37
Body condition score, (-5 d)	3.7	3.8	0.20	0.78
Body condition score change	0.14	0.37	0.08	0.07
Body weight, kg (-8 wk)	740	725	39.0	0.12
Body weight, kg (-5 d)	781	800	39.3	0.91
Body weight change, kg/d	0.8	1.4	0.16	0.03
Calf weight, kg	41.2	41.0	1.73	0.91
Back fat thickness, mm (-14 d) ³	3.7	5.4	1.10	0.21
Back muscle diameter, mm (-14 d) ³	51.3	52.2	2.29	0.65
Postpartum				
Body condition score, +8 wk	2.9	3.1	0.21	0.37
Body weight, kg (+1/2 d)	719	718	39.8	0.98
Body weight, kg (+8 wk)	700	692	18.9	0.50
Body weight change, kg/d	-0.6	-0.8	0.26	0.73
Back fat thickness, mm ^{3,4}	1.12	1.35	0.262	0.36
	(3.1)	(3.9)		
Change in back fat thickness (mm) ⁵	-0.6	-1.0	0.44	0.51
Back muscle diameter, mm ³	45.3	47.8	2.17	0.01
Change in back muscle diameter (mm) ⁵	-9.2	-6.6	1.6	0.28

¹CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

²HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

³Measured with ultrasound on m. longissimus dorsi, pars lumbalis

⁴Values after transformation, back-transformed values are given in parenthesis

⁵The change from d -14 to d +28 relative to parturition.

Table 4. Effect of dry period energy intake and diet composition on blood hormone and metabolite concentration

Item	CEI ¹	HEI ²	SEM	P-value	
				Diet	Diet x Time
Prepartum					
Glucose, mmol/l	3.8	4.0	0.06	0.049	0.06
Log Insulin, μ IU/ml ³	2.67 (14.4)	3.00 (20.1)	0.151	0.07	0.08
1 / Glucagon, pg/ml ^{3,4}	0.0078 (128.2)	0.0071 (140.8)	0.0006	0.45	0.33
Log Glucagon:insulin, mol/mol ^{3,4}	-1.00 (0.37)	-1.03 (0.36)	0.126	0.86	0.60
Log Non-esterified fatty acids, mmol/l	-1.93 (0.15)	-2.09 (0.12)	0.097	0.26	0.07
BHB, mmol/l	0.61	0.67	0.018	0.006	0.27
Log Glycerol, μ mol/L ^{3,5}	3.75 (42.5)	3.83 (46.1)	0.055	0.32	0.93
3-MH, μ mol/l (d -12)	7.48	5.80	0.734	0.09	-
Postpartum					
Glucose, mmol/l	3.2	3.4	0.09	0.09	0.45
Log Insulin, μ IU/ml	2.12 (8.3)	2.22 (9.2)	0.107	0.52	0.37
1 / Glucagon, pg/ml ^{3,6}	0.0069 (144.9)	0.0076 (131.6)	0.0006	0.43	0.37
Log Glucagon:insulin, mol/mol ⁶	-0.33 (0.72)	-0.50 (0.61)	0.142	0.40	0.26
Log Non-esterified fatty acids, mmol/L ³	-1.06 (0.35)	-0.97 (0.38)	0.133	0.62	0.28
1 / BHB, mmol/l	0.76 (1.32)	0.89 (1.12)	0.091	0.32	0.01
Log Glycerol, μ mol/L ³	3.89 (48.9)	3.96 (52.5)	0.074	0.48	0.21
3-MH, μ mol/l ⁷	9.28	8.78	0.819	0.68	0.19

¹CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

²HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

³ Values after transformation, back-transformed values are given in parenthesis

⁴ Sampled on d -56, -12, and d -3 relative to parturition

⁵ Sampled on d -56, -42, -12, -7, -5, -3, and d -1 relative to parturition

⁶ Sampled on d 1, 7, 14, and d 28 relative to parturition

⁷ 3-MH = Plasma 3-methylhistidine concentration sampled on d 1, 7, and d 28 relative to parturition

Table 5. Effect of dry period energy intake and diet composition on milk production responses

Item	CEI ¹	HEI ²	SEM	P-value	
				Diet	Diet x Time
Milk, kg/d	40.1	42.8	1.32	0.18	0.007
ECM, kg/d ³	41.8	44.3	1.21	0.16	0.27
ECM/kg DMI	1.95	2.05	0.08	0.38	0.02
Fat, g/d	1820	1930	61.4	0.22	0.30
Protein, g/d	1300	1380	38.9	0.18	0.05
Lactose, g/d	1770	1830	78.4	0.58	0.01
Milk composition					
Fat, g/kg	47.5	48.3	1.07	0.59	0.003
Protein, g/kg	34.4	34.3	0.51	0.96	0.57
Lactose, g/kg	45.4	45.3	0.29	0.76	0.55
Urea, mg/100 ml	29.0	29.8	1.86	0.72	0.09

¹CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d

²HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements/d

³Energy corrected milk calculated according to Sjaunja et al. (1990).

Table 6. Effect of dry period energy intake and diet composition on plasma glucose, insulin, and non-esterified fatty acids (NEFA) responses to intravenous glucose tolerance test (IVGTT; 0.25 g of glucose/kg of BW) at d 13 prior to parturition and at d 9 postpartum

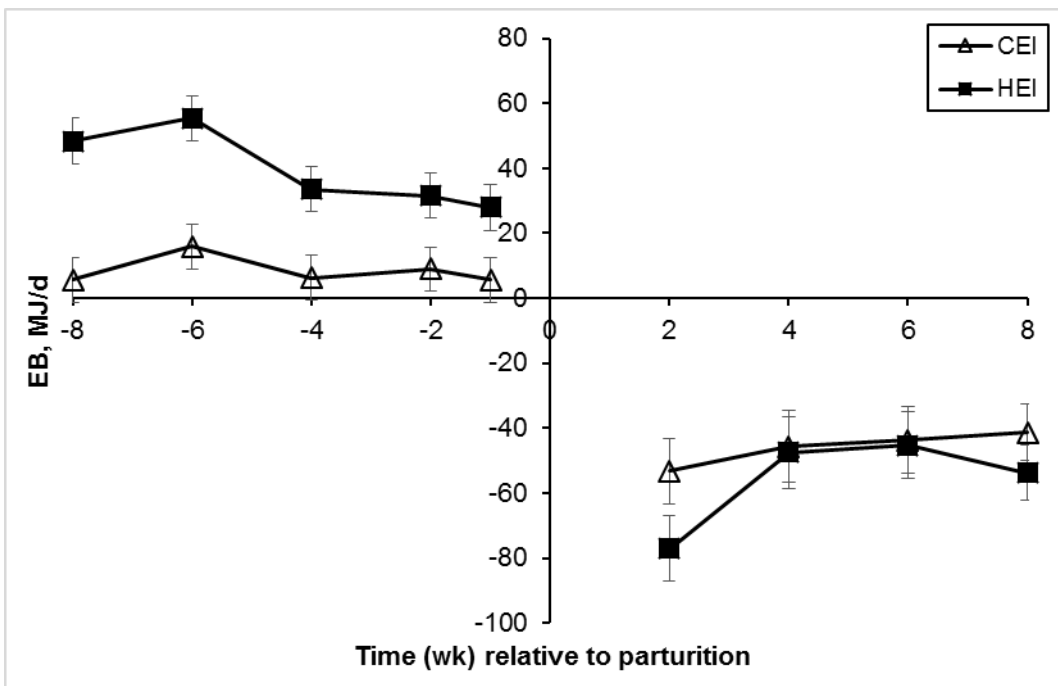
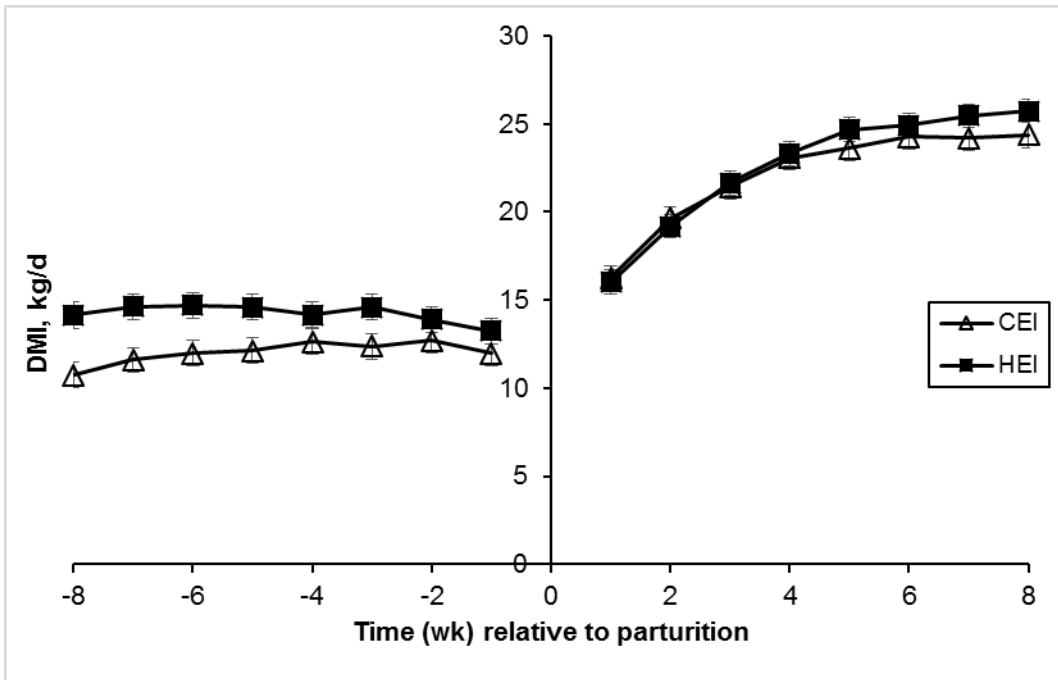
Item ¹	Prepartal IVGTT			<i>P</i>		Postpartal IVGTT			<i>P</i>
	CEI ² (n = 8) ⁵	HEI ³ (n = 8)	SEM	Diet	Day ⁴	CEI ² (n = 8)	HEI ³ (n = 8)	SEM	Diet
Glucose									
Basal (mmol/L)	3.9	4.0	0.12	0.14	0.47	3.0	3.1	0.16	0.51
Peak (mmol/L)	19.4	19.2	0.37	0.71	0.94	15.5	16.8	0.55	0.13
AUC ₆₀ (μIU/mL x 60 min)	404	385	20.0	0.22	0.21	309	294	14.0	0.37
CR ₆₀ (%/min)	1.3	1.4	0.12	0.83	0.93	1.7	2.0	0.17	0.15
AUC ₂₄₀ (mmol/L x 240 min)	525	413	47.9	0.02	0.08	374	322	30.1	0.22
Insulin									
Basal (μIU/mL)	13.8	15.7	2.03	0.52	0.23	6.2	5.7	0.75	0.64
Peak (μIU/mL)	224	398	73.3	0.02	0.007	110	125	17.5	0.56
CR ₆₀ (%/min)	-0.62	-0.72	0.11	0.40	0.20	0.28	0.21	0.13	0.73
AUC ₆₀ (μIU/mL x 60 min)	7920	13916	2622	0.03	0.01	3185	3844	535	0.55*
AUC ₂₄₀ (μIU/mL x 240 min)	10064	17762	3520	0.05	0.02	3063	3884	561	0.30
NEFA									
Basal (mmol/L)	0.29	0.24	0.05	0.12	0.03	0.53	0.62	0.07	0.27
Nadir (mmol/L)	0.10	0.08	0.01	0.32	0.045	0.29	0.28	0.03	0.82
NEFA decrement (mmol/L)	0.19	0.16	0.04	0.19	0.09	0.24	0.33	0.05	0.18
CR ₆₀ (%/min)	1.71	1.25	0.48	>0.10**	-	1.2	1.4	0.24	0.45
AUC ₆₀ (μIU/mL x 60 min)	-2.6	-2.3	1.1	0.83	0.70	-5.2	-7.9	2.05	0.38
AUC ₂₄₀ (mmol/L x 240 min)	-20.0	-13.4	4.33	0.18	0.53	35.0	18.7	17.3	0.53
Minimal model									
SI (x 10 ⁻⁴ min ⁻¹ /μIU/mL)	0.67	0.23	0.31	>0.10**	-	1.74	2.28	0.499	0.40
S _g (min ⁻¹)	0.02	0.03	0.002	>0.10**	-	0.03	0.03	0.002	0.53
AIR _g (μIU/mL/min)	695	981	155	0.002	0.57	456	512	70.5	>0.10*
DI	227	241	93.9	0.92	0.03	825	1065	249	0.51
NEFA model									
FFA ₀ (μmol/L)	390	254	63.5	0.09	0.08	547	641	83.8	0.37
S _{FFA} (μmol/L/min)	19.9	21.5	3.59	0.74	0.86	61.7	75.5	13.5	0.49
K _{FFA} (/min)	0.03	0.04	0.006	0.13	0.59	0.05	0.05	0.009	0.86
Latency (min)	11.9	16.6	1.26	0.04	0.95	10.5	11.4	1.41	0.66

¹ Basal = average concentration at 10 and 5 min before IVGTT; CR₆₀ = clearance rate during the first 60 min of IVGTT; AUC₂₄₀ = area under the curve during 240 min of IVGTT [(mmol/L for glucose and NEFA, μIU/mL for insulin) × 240 min]; AUC₆₀ = area under the curve during the first 60 min of IVGTT; NEFA decrement = basal – nadir; SI = insulin sensitivity index; S_g = glucose effectiveness; AIR_g = acute insulin response to glucose load; DI = disposition index (= AIR_g x SI); FFA₀ = basal NEFA estimated by NEFA model analysis; S_{FFA} = rate of entry of NEFA to the plasma pool; K_{FFA} = rate of removal of NEFA from the plasma pool; Latency = the time until NEFA concentration begin to decline

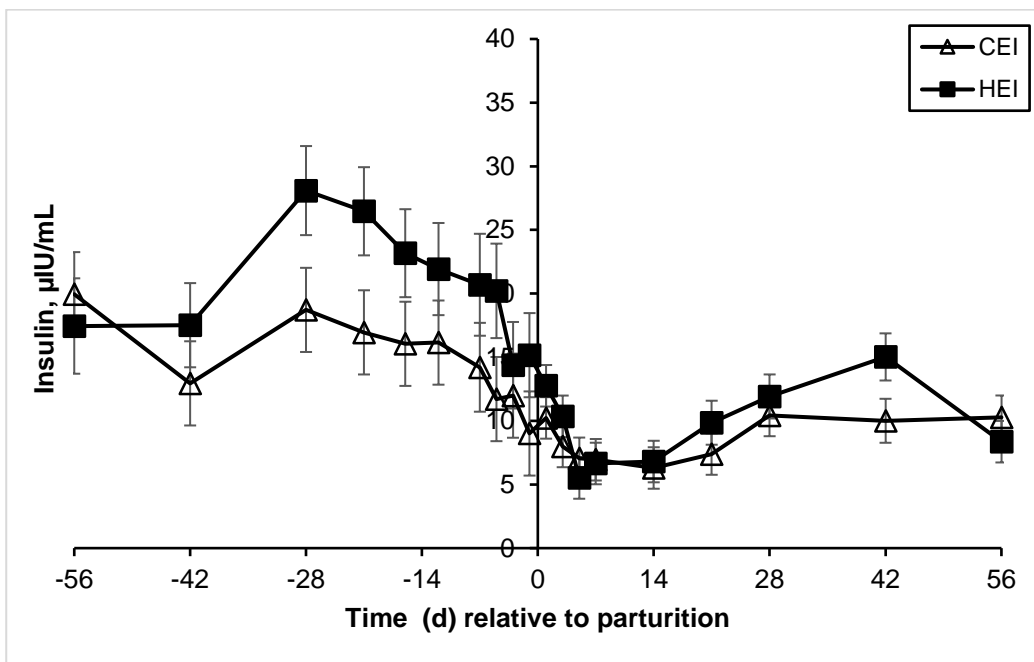
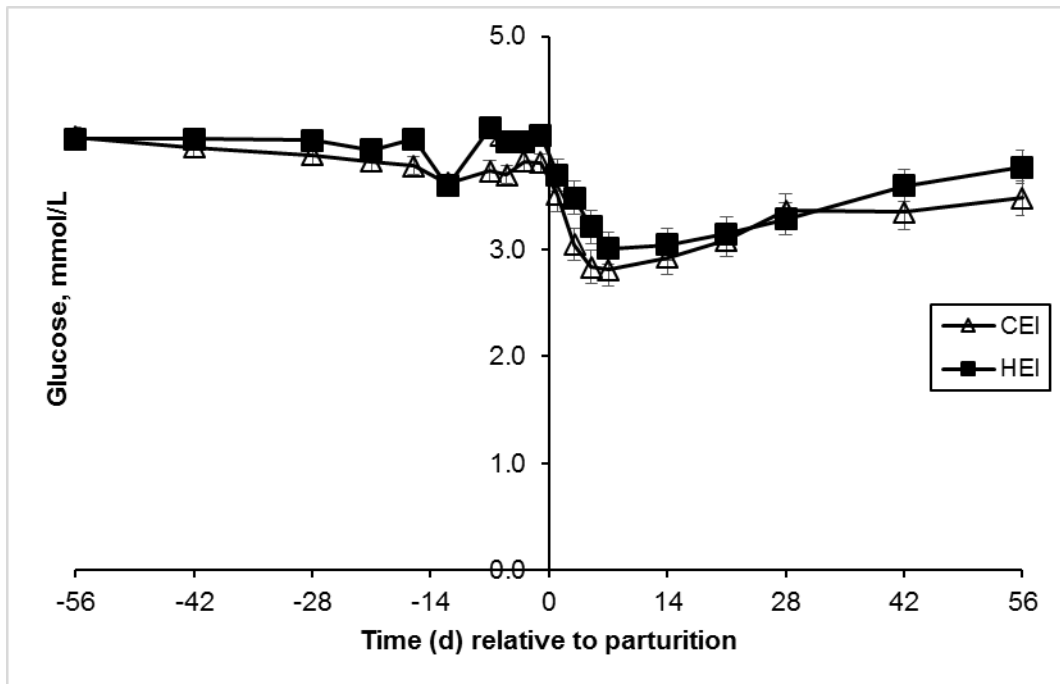
²CEI = Controlled energy intake during 8 wk dry period providing 108% of ME requirements/d; ³HEI = Ad libitum energy intake during 8 wk dry period providing 141% of ME requirements

⁴Day = number of days to expect parturition; ⁵n = 7 for NEFA statistics; *P-values after natural logarithmic transformation; ** P-values from Friedman's non-parametric testing.

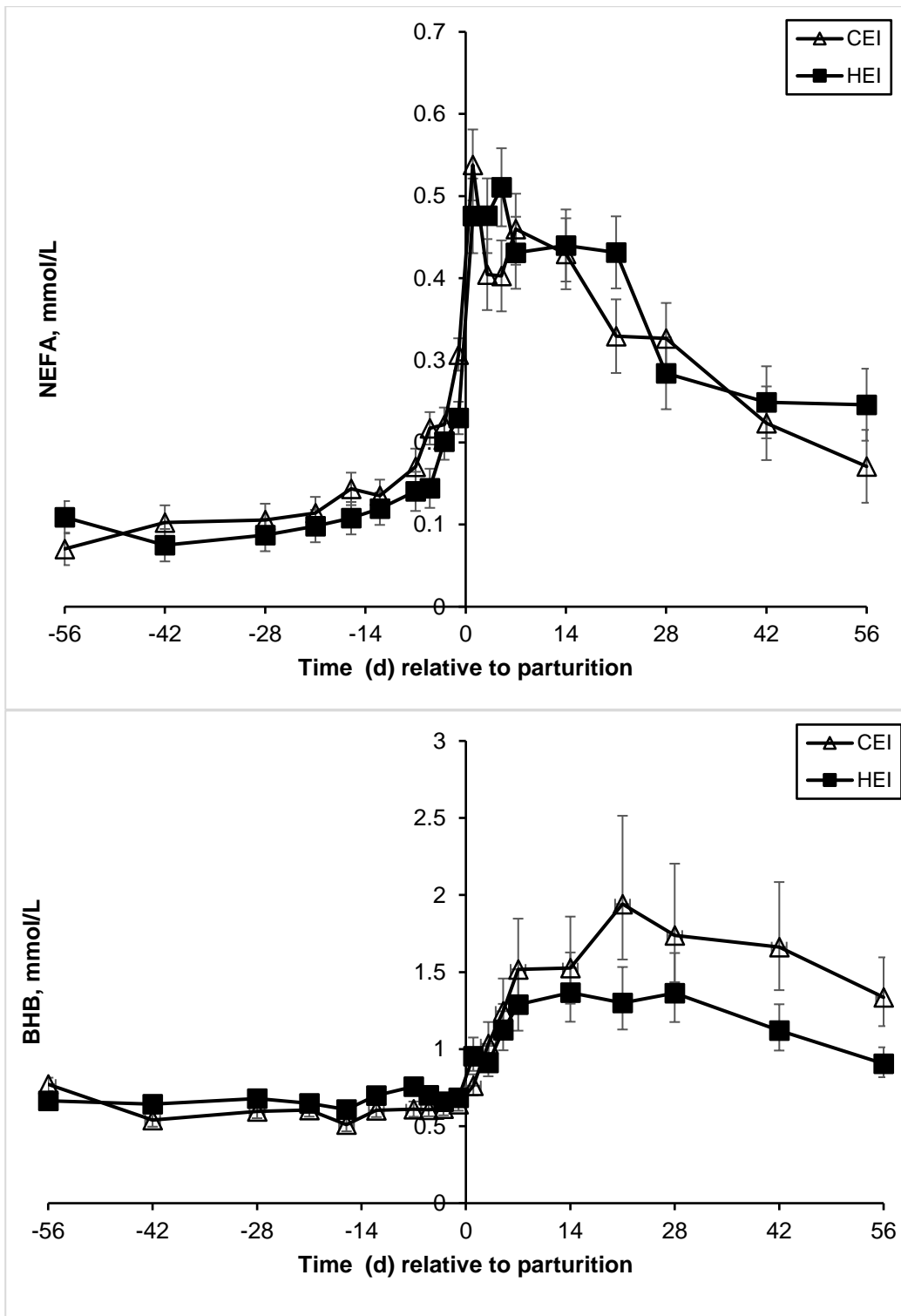
Salin, Figure 1 and 2



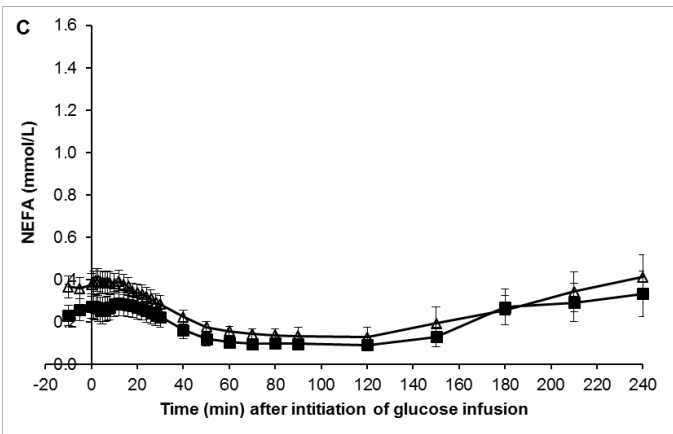
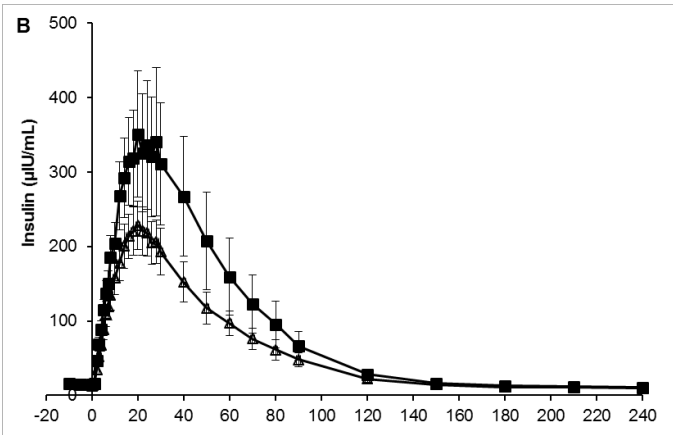
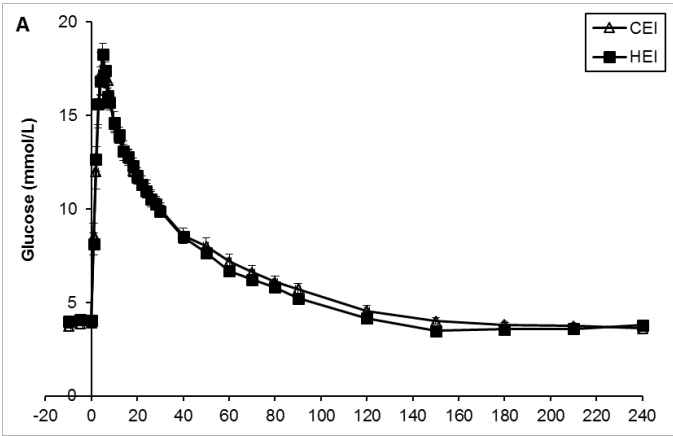
Salin, Figure 3 and 4



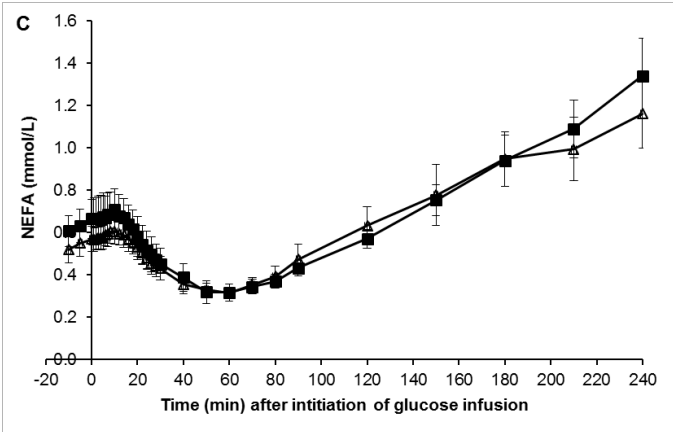
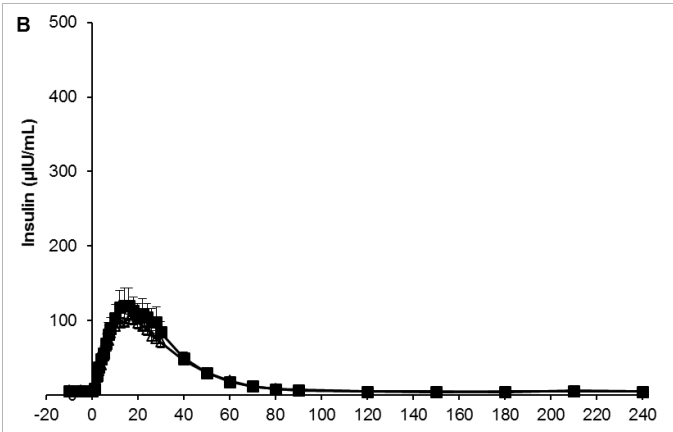
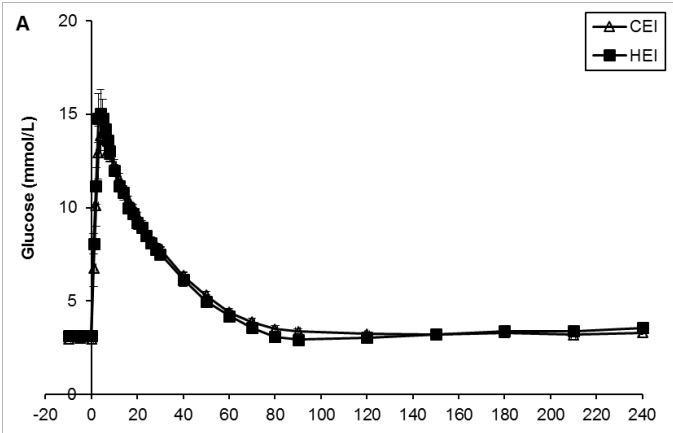
Salin, Figure 5 and 6



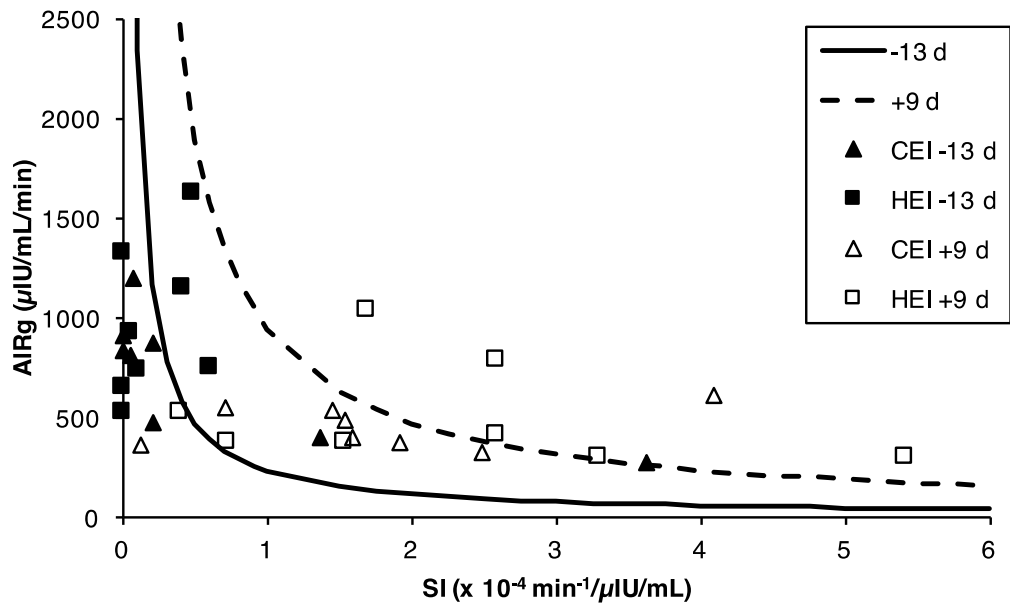
Salin, Figure 7A, 7B, 7C



Salin, Figure 8A, 8B, 8C



Salin, Figure 9



Salin, Figure Captions

Figure 1. Dry matter intake of cows fed two levels of energy in the dry period, CEI (Δ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI (\blacksquare) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are LSM \pm SE of repeated measures analysis (n = 16). Pooled SE pre 0.50 kg/d, postpartum (pp) 0.57 kg/d. Effects of diet pre (P = 0.002), diet x time pre (P = 0.64), diet pp (P = 0.52), diet x time pp (P = 0.57).

Figure 2. Metabolizable energy balance of cows fed two levels of energy in the dry period, CEI (Δ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI (\blacksquare) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are LSM \pm SE of repeated measures analysis (n = 16). Pooled SE pre 4.8 MJ/d, postpartum (pp) 8.7 MJ/d. Effects of diet pre (P = 0.004), diet x time pre (P = 0.51), diet pp (P = 0.46), diet x time pp (P = 0.25).

Figure 3. Plasma glucose concentration of cows fed two levels of energy in the dry period, CEI (Δ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI (\blacksquare) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are LSM \pm SE of repeated measures analysis (n = 16). Effects of diet pre (P = 0.049), diet x time pre (P = 0.06), diet pp (P = 0.09), diet x time pp (P = 0.45).

Figure 4. Plasma insulin concentration of cows fed two levels of energy in the dry period, CEI (Δ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI (\blacksquare) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are back-transformed LSM \pm SE from repeated measures analysis of log-transformed data (n = 16). Effects of diet pre (P = 0.07), diet x time pre (P = 0.08), diet pp (P = 0.52), diet x time pp (P = 0.37).

Figure 5. Plasma NEFA concentration of cows fed two levels of energy in the dry period, CEI (Δ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI (\blacksquare) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Values are back-transformed LSM \pm SE from repeated measures analysis of log-transformed data (n = 16). Effects of diet pre (P = 0.26), diet x time pre (P = 0.07), diet pp (P = 0.62), diet x time pp (P = 0.28).

Figure 6. Plasma BHB concentration of cows fed two levels of energy in the dry period, CEI (Δ) = 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal (55/45/5%); and HEI (\blacksquare) = 141% of the ME requirements of grass silage during wk 8 to 1 prepartum (pre). Prepartal values are LSM \pm SE of repeated measures analysis. Postpartal values are back-transformed LSM \pm SE from repeated measures analysis of reciprocally transformed data (n = 16). Effects of diet pre (P = 0.006), diet x time pre (P = 0.27), diet pp (P = 0.32), diet x time pp (P = 0.01).

Figure 7. Treatment effects on plasma (A) glucose, (B) insulin, and (C) non-esterified fatty acids (NEFA) concentration during intravenous glucose tolerance tests (0.25 g of glucose i.v./kg of BW) performed 13 \pm 5 d before parturition in dairy cows fed 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal [55/45/5%; CEI (Δ)] and in cows fed 141% of the ME requirements of grass silage [HEI (\blacksquare)], during wk 8 to 1 prepartum. Error bars represent SEM. Least squares means of area under the curve for glucose, insulin, and NEFA in CEI and HEI were 525 and 413 \pm 47.9 mmol/L x 240 min, 10064 and 17762 \pm 3520 μ IU/mL x 240 min, and -20.0 and

-13.4 ± 4.3 mmol/L x 240 min (n = 16), respectively. Concentration at time point -5 min represents the average concentration at 10 and 5 min before IVGTT.

Figure 8. Treatment effects on plasma (A) glucose, (B) insulin, and (C) non-esterified fatty acids (NEFA) concentration during i.v. glucose tolerance tests (IVGTT; 0.25 g of glucose i.v./kg of BW) performed 9 ± 1 d after parturition in dairy cows fed 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal [55/45/5%; CEI (Δ)] and in cows fed 141% of the ME requirements of grass silage [HEI (■)], during wk 8 to 1 prepartum. Error bars represent SEM. Least squares means of area under the curve for glucose, insulin, and NEFA in CEI and HEI were 374 and 322 ± 30.1 mmol/L x 240 min, 3063 and 3884 ± 561 μIU/mL x 240, and 35.0 and 18.7 ± 17.3 mmol/L x 240 min, (n = 16), respectively. Concentration at time point -5 min represents the average concentration at 10 and 5 min before IVGTT.

Figure 9. The hyperbolic relationship between the minimal model–derived indices of acute insulin secretion (AIRg) and insulin sensitivity index (SI) denoted as disposition index (DI) during the intravenous glucose tolerance tests (0.25 g of glucose i.v./kg of BW) performed 13 ± 5 d before and 9 ± 1 d after parturition in dairy cows fed 108% of the ME requirements of grass silage, wheat straw, and rapeseed meal [55/45/5%; CEI (Δ)] and in cows fed 141% of the ME requirements of grass silage [HEI (■)], during wk 8 to 1 prepartum. The hyperbolas were generated from extrapolated values of insulin secretion (AIRg) based on the average of observed values of DI for -13 d (n = 16), and +9 d (n = 16), and varying SI in the range from 0.01 to 6 (Stefanovski et al., 2011). All observations of SI and AIRg and the corresponding hyperbolas before and after parturition are represented by the symbols defined in the figure.