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BODY CONDITION EFFECTS ON DRY MATTER INTAKE AND METABOLIC STATUS DURING THE TRANSITION PERIOD IN HOLSTEIN DAIRY COWS

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ABSTRACT. The objective was to evaluate dry matter intake, metabolite concentrations and milk production of cows with different dry period body condition score (BCS). In addition, to support these results with previously reported insulin resistance and adipose tissue mRNA data on the same cows. Multiparous Estonian Holstein cows (n = 42) were assigned to three experimental groups on the basis of BCS 28 days before expected calving (d –28) as follows: BCS ≤ 3.0 (2.25–3.00; thin (T), n = 14); BCS = 3.25–3.5 (optimal (O), n = 14); BCS ≥ 3.75 (3.75–4.50; over-conditioned (OC), n = 14). Blood samples were taken between d –21 and d 42 in relation to calving, milk production data were collected throughout lactation. The OC cows' adaptation to the demands of lactation was the worst based on the comparison of dynamics of blood parameters between BCS groups. They had the most unbalanced metabolism and used more stored lipids compared to T and O cows. Fatty acids concentrations in the first week of lactation, related to insulin resistance status in the dry period and DMI in the first days of lactation, describe most of the variation ($R^2 = 0.55$) in BCS loss during the first 42 days of lactation.

Introduction

The transition period is metabolically the most demanding period throughout the productive period, which is illustrated by high disease incidence and culling rates (Ingvartsen, Moyes, 2013). In order to have trouble-free transitions, the cow needs to successfully adapt to lactation challenges (Gross, Bruckmaier, 2019) and homeorchetic changes during pregnancy play a vital role in the ability to adapt (Bauman, Currie 1980). The main goals for herd managers during the dry-period in supporting good adaption are to avoid overfeeding, overconditioning, and declining dry matter intake (DMI) before parturition (Drackley, Cardoso, 2014).

It has been shown that overfeeding either during the far-off or close-up periods results in higher lipid mobilization postpartum (Dann *et al.*, 2006, Mann *et al.*, 2016). However, the negative effect of overfeeding seems to be more pronounced with diets high in starch and its residues as studies with grass-based feeding have not given similar results (Agenäs *et al.*, 2003, Salin *et al.*, 2018). Long term overfeeding, irrespective of diet, will lead to increased fat reserves as the excess of dietary energy is mainly stored in the adipose tissue. Body condition score (BCS) is a good proxy for the amount of fat reserves, and over-conditioned cows face a greater risk of high lipid mobilization leading to high risk for health problems, while under-conditioned cows produce less milk and have lower fertility (Roche *et al.*, 2009). The range of optimum body condition is still debated and may depend on a genotype as cows with higher genetic merit or milk production are prone to a higher rate of lipolysis compared to lesser peers (Khan *et al.*, 2013, Karis *et al.*, 2020). Adequate intake during the transition period indicates cows' adaption to lactation and leads to reduced negative energy balance (NEB). Both overfeeding and overconditioning are associated with lower DMI postpartum (Drackley, Cardoso, 2014).

At the beginning of lactation cows' endocrine status favours lipid mobilization mainly through adrenergic signalling and the blunted effect of its main antagonist insulin on adipocytes will further induce lipolysis. This

situation of insulin resistance (IR) is deemed to develop during the dry period (De Koster, Opsomer, 2013). We have previously shown that the development of IR, changes in the expression of mRNAs and proteins related to glucose and lipid metabolism are dependent on the body condition in the dry period (Jaakson *et al.*, 2018, Karis *et al.*, 2020). In this study, we set out to test the hypothesis that the factors through which the amount of fat reserves before calving affects cows' adaption to lactation are interrelated. Our objective was to evaluate DMI around calving, concentrations of metabolites related to a potential overload of metabolism, and milk production of cows with different dry period BCS.

Material and methods

Experimental design

The European Council Directive regarding the protection of animals and the Estonian Animal Protection Act have been complied with in this experiment. The study was approved by the Committee for Conducting Animal Experiments at the Estonian Ministry of Rural Affairs. The study was carried out over two consecutive years (2013–2015) on the experimental farm of the Estonian University of Life Sciences with a herd size of 120 cows and a mean annual milk yield of 9 200 kg cow^{-1}.

Cows BCS was assessed fortnightly according to the method described by Edmonson *et al.* (1989) starting from dry-off approximately 60 days before expected calving. Cows whose BCS 28 days before expected calving (d –28) was the same as at dry-off were eligible for the study. In total, 46 Estonian Holstein cows were assigned to three experimental groups on the basis of BCS on d –28 as follows: BCS ≤ 3.0 (2.25–3.00, thin (T)); BCS 3.25–3.5 (optimal (O)); BCS ≥ 3.75 (3.75–4.50, over-conditioned (OC)). Due to culling in the first two weeks of lactation, 4 cows were excluded from the study. The remaining 42 cows were equally distributed between three experimental groups, 14 each. The average parity of the cows after calving during the trial period was the following: T – 2.6 ± 0.9 (parity 2 [n = 9], 3 [n = 3], 4 [n = 1], 5 [n = 1]), O – 3.2 ± 1.2 (parity 2 [n = 5], 3 [n = 4], 4 [n = 3], 5 [n = 1], 6 [n = 1]), OC – 3.7 ± 1.0 (parity 2 [n = 1], 3 [n = 6], 4 [n = 4], 5 [n = 2], 6 [n = 1]). Cows were enrolled in accordance with blocked design with each block consisting of three cows, one from each experimental group. Fortnightly BC scoring was continued until d 42 postpartum.

On d –28 cows were removed from the dry cow barn to tie-stall housing and from the seventh milking, cows were moved to a free-stall barn with a milking parlour.

The cows were milked twice daily at 05:00 and 15:00. Milking parlour hardware (DeLaval, Tumba, Sweden) recorded milk yields. Morning and evening milk samples were collected either on Sundays and Thursdays (from March 2013 to April 2014) or on Sundays, Thursdays and Tuesdays (from May 2014 to December 2015) with in-line milk meters (DeLaval, MM27BC, Sweden). Milk samples were stabilized with bronopol (Broad Spectrum Microtabs, D&F Control Systems Inc., Norwood, MA) and were analysed for fat and protein with an automatic infrared milk analyser (System FT+, Foss Electric, Hillerød, Denmark) in the Milk Analysis Laboratory of Estonian Livestock Performance Recording Ltd. ECM-yields were calculated according to Sjaunja *et al.* (1990). A fourth-order polynomial was fitted to milk, ECM, milk fat and milk protein production values. The area under the curve was calculated for each of the variables as the definite integral of the fitted polynomial and used as an estimate for total production over 42 and 305 DIM.

Disease events during the trial period are reported elsewhere (Jaakson *et al.*, 2018), these disease events were not taken into account in the current study. In addition, three cows from group T and three cows from group OC were culled before the end of lactation. The culling reasons were the following: two incidences of feet disease and four incidences of udder disease.

We have previously reported data on glucose tolerance test carried out on d –21 and d 21, data on mRNA and protein abundance in subcutaneous adipose tissue taken on d –21 and d 21 of the same cows (Jaakson *et al.*, 2018, Karis *et al.*, 2020). Blood samples were taken approximately one hour before the start of the glucose tolerance test.

Feeding, DMI drop, BCS loss

Diets, ingredients and chemical composition are presented in table 1. Cows were fed TMR *ad libitum* twice a day, at 05:30 and 14:30. Diets were calculated according to Estonian feeding recommendations: metabolizable energy (ME) according to Oll and Tölp (1995), metabolizable protein (MP) as described by Kärt *et al.* (2002). Between d –28 to d 2 cows were fed individually and orts were collected and weighed twice daily before the fresh feed was offered. Daily intake was calculated as the difference between the weight of feed offered and the weight of orts. To calculate the DMI drop average DMI between d –14 to d –8 were calculated for each cow, which was thereafter subtracted from DMI on d –1.

Third-order polynomial was fitted to BCS data, and BCS loss was calculated as the difference between the model's value on d 42 minus the value on d 1.

Blood sampling and laboratory analyses

Blood samples were taken on d –21 ± 2.3, d –14 ± 2.1, d –7 ± 2.6, d 7 ± 0.9, d 14 ± 0.8, d 21 ± 1.0, d 28 ± 1.1 and d 42 ± 2.1 at around 10:00 from the coccygeal vein into vacuum tubes containing Li-heparin (VACUETTE®, Greiner Bio-One International GmbH, Kremsmünster, Austria). Samples were centrifuged (5 000 × g, 15 min, +4°C) and stored at –80 °C. Clinical chemistry analyser (ERBA XL300, ERBA Diagnostics Mannheim GmbH, Mannheim, Germany) was used to measure the concentration of plasma fatty acids (also known as non-esterified fatty acids (NEFA)) (cat. no. FA 115; Randox Laboratories Ltd., Crumlin, United Kingdom), β-hydroxybutyrate (BHB) (cat. no. RB 1007; Randox Laboratories Ltd.),

total antioxidant status (TAS) (cat. no. NX 2332; Randox Laboratories Ltd.), aspartate aminotransferase (AST) (product code XSYS016, ERBA Diagnostics Mannheim GmbH, Germany), glucose (product code XSYS012, ERBA Diagnostics Mannheim GmbH), albumin (product code XSYS001 ERBA Diagnostics Mannheim GmbH), blood urea nitrogen (BUN) (product code XSYS020 ERBA Diagnostics Mannheim GmbH), uric acid (product code BLT00062 ERBA Diagnostics Mannheim GmbH). All of the inter- and intra-assay CVs were below 8.3 and 6.1%, respectively.

Insulin was analysed from the same eight samples by bovine-optimized sandwich ELISA (cat no 10-1201-01; Mercodia AB, Uppsala, Sweden), with a detection limit of 0.025 ng mL^{-1}, on a microplate reader (SunriseTM, Tecan Group Ltd., Switzerland); results were calculated using cubic spline regression (Magellan™ data analysis software; Tecan Group Ltd., Switzerland). The inter-assay coefficients of variation for plasma insulin concentrations of 0.2 ng mL^{-1} and 1.6 ng mL^{-1} were 4.6 and 6.6%, respectively and the intra-assay coefficients of variation were 4.2 and 4.1%, respectively.

Statistical analysis

Statistical analyses were performed with software R (version 3.5.0, R Foundation of Statistical Computing, Vienna, Austria). The DMI data from d –28 to d –15 and from d –14 to d –8 were averaged for each cow. To estimate the effect of the BCS group and time on the dependent variables a mixed linear model was fitted, considering the fixed effects of the BCS group, time, their interaction and the confounding factors of parity and relative breeding value for milk production, and random effects of animal and block. Pre- and post-partum blood sample data were modelled separately with the exception of DMI between d –7 to d 2 in relation to calving. For variables that were measured once or pooled, the fixed effect of time and random effect of the animal was removed from the model. If the modelling resulted in a singular fit due to the effect of a block being close to zero it was removed from the model. Modelling was performed with the function "lmer" in the package "lme4" and the least square means (LSM, *alias* marginal or model-based means) were estimated with the function "emmeans". The pairwise comparison of LSM was performed with the function "contrast". P-values were adjusted for multiple testing with the Tukey method. The significance of the effect of factors considered in the model was estimated with the type 2 Wald Chi-Square Test with the function "Anova". Two analyses were performed for variables that were not normally distributed (normality tested with the Kolmogorov-Smirnov test): at first, the models were fitted and the LSM were estimated on an arithmetic scale, and subsequently, the P-values were estimated fitting the same models on logarithm-transformed values. Linear regression lines and coefficients of determination were calculated to investigate the relationships between fatty acids on d 7 and DMI on d 2, between fatty acids on d 7 and BCS loss. Statistical significance was declared at $P < 0.05$.

Table 1. Diets ingredients and chemical composition (Mean ± SD) of total mixed rations diets (published in Jaakson *et al.*, 2018 and Karis *et al.*, 2020)

Item	Diets				
	Far-off Until d –15	Close-up d –14 to –1	Lactation 1 d 1 to 6	Lactation 2 d 7 to 14	Lactation 3 from d 15
Ingredient, g kg^{-1}					
Grass silage	955 ± 81	599 ± 71	604 ± 72	460 ± 49	384 ± 37
Hay	33.6 ± 81	28.3 ± 69	28.6 ± 70	20.3 ± 49	15.6 ± 37
Barley meal	–	301 ± 10	303 ± 10	309 ± 0.1	296 ± 0.2
Cornmeal	–	–	–	64.3 ± 0.0	120 ± 0.1
Heat-treated rapeseed cake	–	47.1 ± 0.6	47.5 ± 0.6	129 ± 0.0	168 ± 0.1
Mineral-vitamin feed	11.7 ± 0.11	7.82 ± 0.21	10.5 ± 0.32	8.58 ± 0.02	7.99 ± 0.02
Anionic mineral feed[3]	–	10.4 ± 0.2	–	–	–
Limestone	–	6.26 ± 0.2	–	4.29 ± 0.0	4.41 ± 0.7
Sodium chloride	–	–	5.78 ± 0.1	4.72 ± 0.0	4.40 ± 0.0
DM of diet	348 ± 78	435 ± 64	433 ± 64	483 ± 53	511 ± 45
Chemical composition					
CP, g kg^{-1} of DM	131 ± 10.5	144 ± 7.2	145 ± 7.3	161 ± 5.6	169 ± 4.8
MP, g kg^{-1} of DM	72.8 ± 3.3	86.6 ± 2.1	87.3 ± 2.1	97.9 ± 1.6	104 ± 1.3
ME, MJ kg^{-1}	8.70 ± 0.3	10.1 ± 0.2	10.2 ± 0.2	10.9 ± 0.1	11.3 ± 0.1
NDF, g kg^{-1} of DM	534 ± 51	455 ± 32	458 ± 32	410 ± 25	380 ± 22
ADF, g kg^{-1} of DM	387 ± 47	278 ± 29	280 ± 29	242 ± 22	220 ± 18
Ca, g kg^{-1} of DM	11.4 ± 1.9	10.3 ± 1.3	9.33 ± 1.3	9.54 ± 1.0	8.97 ± 0.8
P, g kg^{-1} of DM	3.42 ± 0.4	4.06 ± 0.2	4.03 ± 0.2	4.48 ± 0.2	4.77 ± 0.2

[1] Composition (as-fed basis): 170 g kg^{-1} of Ca; 50 g kg^{-1} of P; 30 g kg^{-1} of Na; 140 g kg^{-1} of Mg; 30 g kg^{-1} of S; 1000 mg kg^{-1} of Cu; 4500 mg kg^{-1} of Zn; 4000 mg kg^{-1} of Mn; 40 mg kg^{-1} of Se; 50 mg of Co; 200 mg of I; 800 000 IU kg^{-1} of vitamin A; 190 000 IU kg^{-1} of vitamin D; and 8000 IU kg^{-1} of vitamin E.

[2] Composition (as-fed basis): 150 g kg^{-1} of Ca; 35 g kg^{-1} of P; 75 g kg^{-1} of Na; 90 g kg^{-1} of Mg; 1 g kg^{-1} of S; 4000 mg kg^{-1} of Cu; 6667 mg kg^{-1} of Zn; 6452 mg kg^{-1} of Mn; 94 mg kg^{-1} of Se; 109 mg of Co; 650 000 IU kg^{-1} of vitamin A; 150 000 IU kg^{-1} of vitamin D; and 4000 IU kg^{-1} of vitamin E.

[3] Composition (as-fed basis): 9 g kg^{-1} of Ca; 1 g kg^{-1} of P; 5 g kg^{-1} of Na; 100 g kg^{-1} of Mg; 1000 mg kg^{-1} of Cu; 5000 mg kg^{-1} of Zn; 2000 mg kg^{-1} of Mn; 27 mg kg^{-1} of Se; 40 mg of Co; 100 mg of I; 1 000 000 IU kg^{-1} of vitamin A; 60 000 IU kg^{-1} of vitamin D; and 10 000 mg kg^{-1} of vitamin E, 100 000 mcg kg^{-1} of biotin.

Results

Group differences in milk yield, DMI and BCS loss

Milk yield for the first 42 DIM, 305-day milk and ECM production did not differ between the groups, but ECM production up to d 42 was greater in group OC compared to group T ($P < 0.05$; Table 2). OC cows had a higher milk fat percentage in the first 42 days of lactation compared to O ($P < 0.05$) and T ($P < 0.01$) cows.

DMI from d –14 to d –8 was lower in OC cows compared to T cows ($P < 0.05$; Table 2). In addition, OC cows DMI were lower than those of T cows on d –3 and d –1 ($P < 0.05$; Fig. 1). On d 1 and 2 postpartum DMI was the greatest in T cows ($P < 0.01$). A DMI drop was significant for all groups (T – $P < 0.05$; O and OC – $P < 0.01$), but it did not differ between groups.

The BCS on d –28 was different between the groups ($P < 0.01$) and the loss until 42 DIM was the greatest in OC cows ($P < 0.01$).

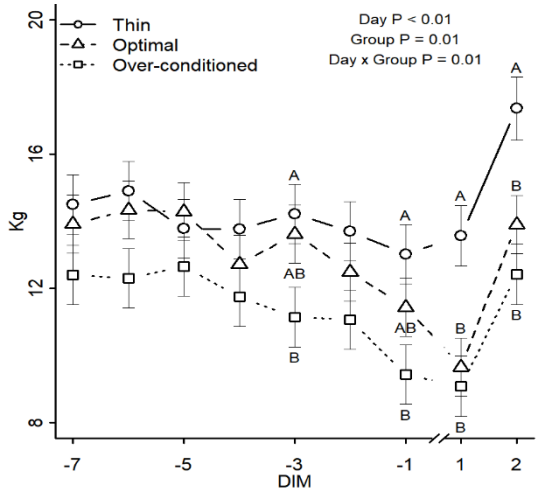

Figure 1. Dry matter intake around calving in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference ($P \leq 0.05$)

Group differences in blood metabolites

Throughout the study period OC cows had a higher concentration of fatty acids, differing from T and O cows on d –28 and d –14, and from T cows on d –7, d 7 and d 21 ($P < 0.05$; Fig. 2). The BHB concentration was lowest on d –14 for OC cows and higher than in T cows on d 14 ($P < 0.05$). On d –21 the glucose concentration in T cows was lower than in OC cows ($P < 0.05$; Fig. 3). From pre- to postpartum glucose and insulin followed the same dynamics. No differences were recorded in the concentrations of insulin between the BCS groups. The activity of AST was highest in OC cows on d 7 ($P < 0.05$), on d 14 ($P < 0.01$), and higher than in T cows on d 21 ($P < 0.05$; Fig. 4).

Albumin concentration was higher prepartum in OC cows compared to T cows ($P < 0.01$), in addition, it was higher than O cows on d –14 ($P < 0.01$; Fig. 5). No differences were recorded postpartum. No differences were also found in TAS throughout the experimental period. Uric acid concentration differed only on d –21, at which point OC cows had greater concentrations compared to T cows (Fig. 6). The BUN concentration did not differ between groups prepartum, but in the postpartum period, T cows had the highest concentration on d 21 (compared to O, $P < 0.05$; compared to OC, ($P < 0.01$)) and higher than OC cows on d 28 ($P < 0.05$).

Associations between DMI, BCS loss and blood fatty acids

In the calculation of regression lines and coefficients of determination, the BCS groups were not differentiated. There was a negative correlation between fatty acids on d 7 and DMI on d 2 and a positive correlation between fatty acids on d 7 and BCS loss during the first 42 DIM (Fig. 7). DMI on d 2 described 37% of fatty acids variance on d 7 and fatty acids on d 7 described 55% of BCS loss variance ($P < 0.01$).

Table 2. Milk yield and composition, DMI, and body condition score (BCS) characteristics. Values are expressed as LSM. Letters "a", "b" and "c" indicate a difference ($P \leq 0.05$)

Characteristic	Group			SEM	P-value
	Thin	Optimal	Over-conditioned		
Milk					
up to d 42, kg d^{-1}	36.0	36.7	38.0	54.4	0.573
up to d 305, kg	10 079	10 639	10 824	463	0.495
ECM[1] up to d 42, kg d^{-1}	37.1^{a}	38.6^{ab}	42.7^{b}	56.2	0.019
ECM up to d 305, kg	9 901	10 484	10 909	384	0.133
Fat up to d 42, %	4.25^{a}	4.38^{a}	4.99^{b}	0.15	0.000
Fat up to d 305, %	3.89	3.87	4.02	0.14	0.717
Protein up to d 42, %	3.32	3.36	3.33	0.06	0.816
Protein up to d 305, %	3.22	3.31	3.34	0.06	0.308
DMI, kg					
d –28 to d –15	14.2	13.8	12.2	0.72	0.126
d –14 to d –8	15.1^{a}	14.8^{ab}	12.7^{b}	0.68	0.017
drop[2]	1.77	3.52	3.46	0.85	0.235
BCS					
on d –28	2.86^{a}	3.33^{b}	3.89^{c}	0.06	0.000
loss[3]	0.45^{a}	0.64^{a}	1.07^{b}	0.08	0.000

[1] Energy corrected milk, calculated according to Sjaunja *et al.*, 1990.
[2] The subtract between the average DMI on d –14 to d –8 and d –1.
[3] Total BCS loss on the first 42 days in milk.

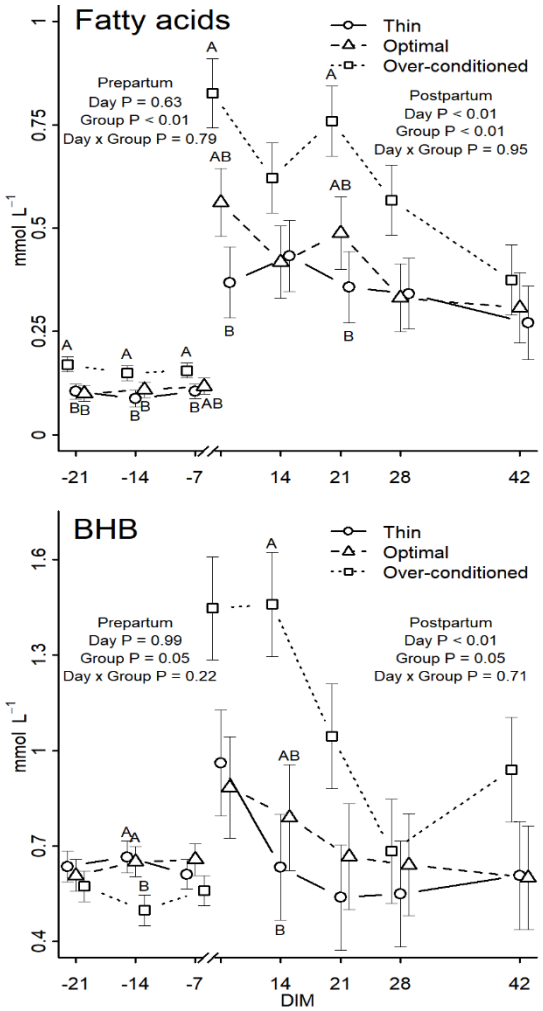

Figure 2. Concentrations of fatty acids and β-hydroxybutyrate (BHB) during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

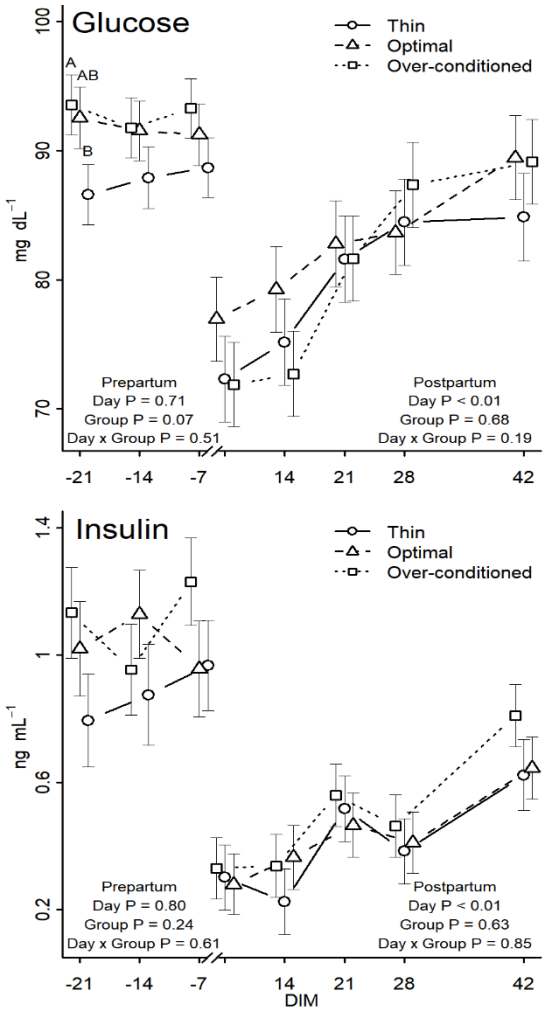

Figure 3. Concentrations of glucose and insulin during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

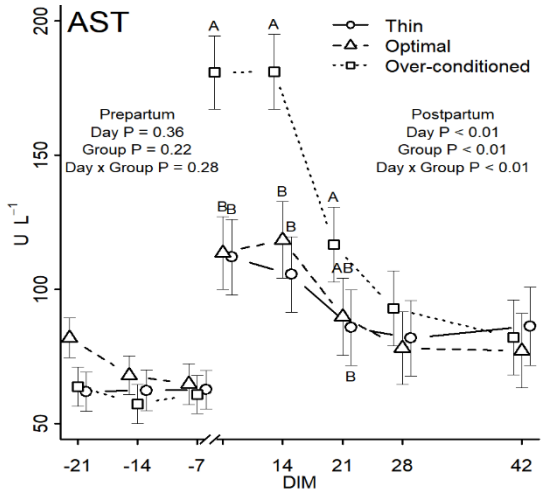

Figure 4. The activity of aspartame aminotransferase (AST) in plasma during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

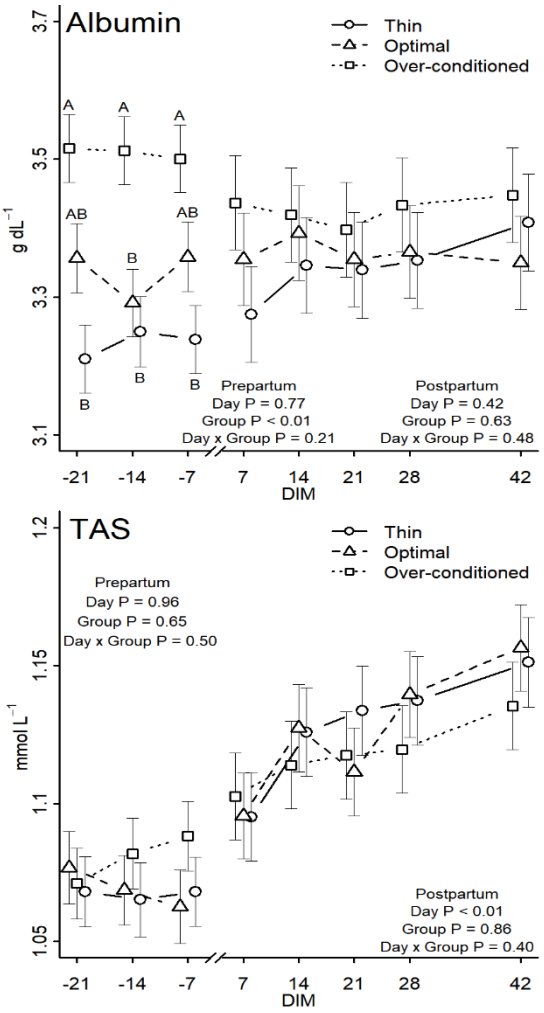

Figure 5. The concentration of albumin and total antioxidant status (TAS) during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

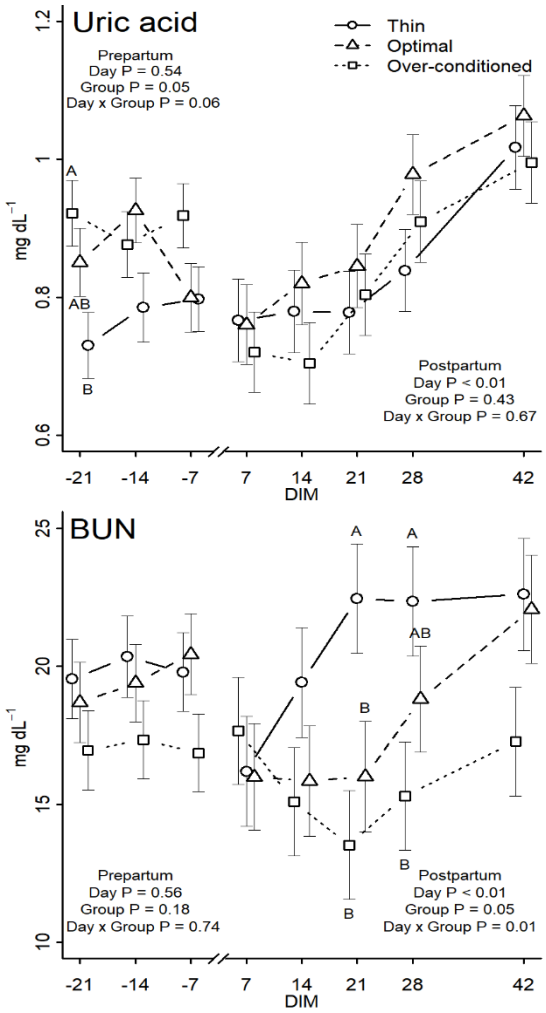

Figure 6. The concentrations of uric acid and blood urea nitrogen (BUN) during the experimental period in multiparous Holstein cows grouped according to BCS on d –28 as follows: ≤ 3.0 – Thin; 3.25–3.5 – Optimal; ≥ 3.75 – Over-conditioned (n = 14 each). Values are expressed as LSM ± SEM. Letters "A" and "B" indicate a difference (P ≤ 0.05)

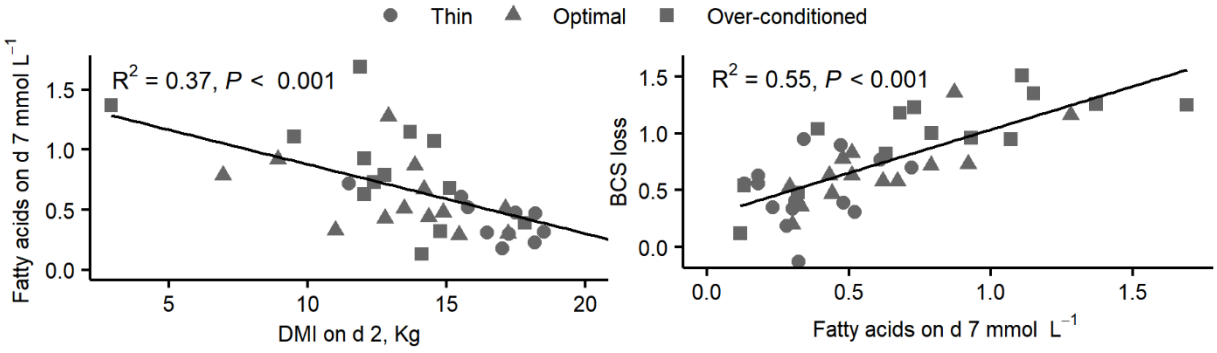

Figure 7. Regression line and coefficient of determination (R^2) of dry matter intake (DMI) on d 2 postpartum and body condition score (BCS) loss during first 42 days in milk with plasma fatty acids concentration on day 7. Points represent the values of individual multiparous Holstein cows

Discussion

This paper is a companion paper to that of Jaakson *et al.* (2018) and Karis *et al.* (2020) and proposes an integration of those findings with longitudinal dynamics in DMI, BCS, blood metabolite concentrations and milk composition of the same cows.

A limitation of our study is the uneven distribution of parity between the experimental groups. To compensate, we added the effect of parity to each mixed linear model as a confounding factor. In most cases, the effect

of parity was small and insignificant; the only exception is DMI between d –28 to d –15.

The magnitude of change in most of the concentrations of the measured metabolites illustrates the drastic shift in cows' metabolism in order to adapt to lactation.

The effect of BCS on DMI, milk and blood biomarkers

Greater glucose concentrations on d –21 and fatty acids concentrations throughout the prepartum period in OC cows compared to T cows might be a reflection of IR, as we have previously reported that OC cows had greater glucose and insulin AUC (Jaakson *et al.*, 2018) as well as longer fatty acids latency (Karis *et al.*, 2020) after glucose infusion on d –21. The differences in glucose concentrations between groups disappeared after d –21 probably because the DMI of OC cows was lower between d –14 to d –8 compared to T cows, and diet starch concentrations were increased two weeks before calving.

We observed differences in uric acid and albumin concentrations prepartum, but the physiological causes and meaning remain uncertain. Albumin serves as a marker of liver activity, inflammation, protein degradation or undernutrition (Agneäs *et al.*, 2003; Bertoni, Trevisi, 2013), but neither of those seems plausible explanations for these cows prepartum. Roche *et al.* (2013) also found a tendency for lower albumin in thin cows prepartum. The difference in uric acid, a marker for microbial protein degradation in the intestine that correlates with DMI (Tas, Susenbeth, 2007), is counter-intuitive as its concentration was lower for T cows even though the DMI was higher on d –28 to d –15 (though, no significance was observed).

It has become common knowledge that a dairy cow's DMI gradually decreases up to 30% over the last three weeks of gestation (Ingvartsen, Andersen, 2000). However, our findings do not support this notion as we observed a DMI drop only in the final days of gestation, and this seemed to be dependent on BCS as we saw a slight DMI drop in T, but a noticeable drop in O and OC cows in the final days of gestation. In agreement with our results, Agenäs *et al.* (2003) and Salin *et al.* (2018) did not report a depressed DMI during close-up in cows overfed with grass silage. High starch intake seems to be one of the possible reasons for DMI depression prepartum (Grummer *et al.*, 2004, Roche *et al.*, 2013). The reason could be the fact that glucose, availability of which is increased, is more tightly regulated and has more of an effect on the endocrine system (Bradford, Allen, 2007). We hypothesize that grass-based diets in the dry period protect cows from a gradual DMI drop. This is also consistent with the hepatic oxidation theory for DMI as acetate, the more dominant volatile FA in grass silage based diets is not extracted from the blood by the liver (Allen, 2020).

The DMI drop for O and OC cows resulted in a difference between T cows in the first two days of lactation. Regarding the T and O groups, this difference was not reflected in the concentrations of blood metabolites as we only recorded differences on BUN concentrations on d 21 between the groups postpartum. Our DMI data end at d 2, thus there is a 5-day discrepancy between the first postpartum blood samples. We hypothesize that the DMI of O cows must have caught up with that of the T cows. This is supported by the facts that we previously reported no difference in the total energy balance during the first 21 DIM (Jaakson *et al.*, 2018) for the same cows nor a difference in body condition loss between groups T and O.

Even though OC cows' fatty acids and BHB concentrations postpartum differed only from the T cows, we conclude that OC cows had the highest lipolysis up to the third week of lactation. We argue that the excess of fatty acids released from the adipose tissue of OC cows was divided between compartments within the organism (*e.g.* liver, udder) and synthesized into other metabolites (*e.g.* BHB), thereby lowering fatty acids concentration in plasma. This is supported by the very high BHB concentrations on d 7 and d 14 in OC cows and the highest total ECM production during the first 42 DIM, which is driven mainly by a high-fat content in milk. In the first week of lactation, the uptake of fatty acids from the blood by the mammary gland accounts for approximately 40% of total milk fatty acids (Bell, 1995). Probably the OC cows' high milk fat percentage, particularly in the first week of lactation, is caused by high lipolysis and fatty acids concentrations in the blood. This is supported by the highest C18:1 *cis*-9 fatty acid concentration in the milk ($P < 0.01$, unpublished data) and the greatest BCS loss in OC cows during six weeks of lactation.

The high activity of AST in OC cows agrees with other metabolites and milk traits data indicating again high lipolysis in OC cows that put them at risk of metabolic and infectious disease (Ingvartsen, Moyes, 2013). Over-conditioned cows are known to be at risk for fat infiltration to liver cell and AST activity is one of its indicators (Bobe *et al.*, 2004). In agreement with his, low urea is also associated with increased TAG in the liver (Jorritsma *et al.*, 2001) and lower expression of some mRNA encoding of enzymes involved in ureagenesis in the liver for cows at risk of high lipid mobilization (Graber *et al.*, 2010). Pires *et al.* (2013) reported evidence indicating higher labile protein mobilization in thin cows, presumably to compensate for the lack of energy from adipose tissue, thus it is plausible that the highest BUN for T cows on d 21 may arise from the differences in protein degradation in addition to liver function.

Increasing TAS postpartum shows that dairy cows are challenged with high rates of oxidation, which is caused by ongoing metabolic stress and NEB. This leads to increased synthesis of reactive oxygen species that may overwhelm the antioxidant capacity and lead to oxidative stress and inflammation response (Sordillo, Raphael, 2013). Even though high lipid mobilization (Sordillo, Raphael, 2013) and high BCS (Bernabucci *et al.*, 2005) are associated with systemic oxidative stress and inflammation, we observed no

differences in TAS or albumin between the BCS groups postpartum. There are more specific biomarkers for inflammation and oxidative stress, for example, tumour necrosis factor α and glutathione peroxidase, than used in this study and tissue-specific alterations might occur (*e.g.* adipose tissue inflammation) (Contreras *et al.*, 2018), therefore a definitive decision on this cannot be made.

Association between DMI, fatty acids and BCS loss

DMI on d 2 describes 37 per cent of the variation of fatty acids on d 7, which in turn seems to be the major determinant for the overall BC loss over the first 6 weeks of lactation. Thus, the success of the transition period is determined in early lactation and relies on the adaption of cows metabolism to a new physiological state during the dry period. In agreement, according to the hepatic oxidation theory, increased plasma fatty acid concentration, and thus intensified oxidation in the liver, is the limiting factor for DMI during early postpartum (Allen, Piantoni, 2013) and improving cows DMI intake pre- and postpartum is seen as the main goal to ensure a trouble-free transition period (Drackley, Cardoso, 2014). De Koster *et al.* (2019) clustered cows based on glucose, fatty acids, BHB and insulin-like growth factor 1 concentrations in blood at the beginning of lactation and reported that the main differences between metabolically balanced and unbalanced cows are DMI and BCS loss postpartum. Our data show that in addition to unfavourable metabolite concentrations OC cows had lower DMI compared to T cows and greater BCS loss than T and O cows and therefore can be classified as metabolically unbalanced. Although, DMI between OC and O cows did not differ on d 1 and 2 postpartum, OC cows still mobilize more body lipids, especially in the first weeks of lactation, suggesting that there are more factors determining the balance of metabolism or, in other words, OC cows experience higher lipolysis regardless of DMI. As only two days of postpartum DIM data was available in our study, and it might not reflect the actual feed intake during early lactation, our interpretation must be taken with caution. The underlying reason for higher lipolysis might be IR, as we have shown its interaction with body condition, and that cows with greater insulin AUC prepartum have decreased lipogenesis potential (lower *LPL* and *DGAT2* mRNA abundance) and greater fatty acids concentration on d 7 (Karis *et al.*, 2020). With good management practices the BCS of dry off cows can be optimized, but the reason for IR prepartum needs to be further studied, including the role of a genetic component in its development.

Conclusions

Overconditioning causes minor differences in glucose and fatty acids concentrations during the last three weeks of prepartum. During the first six postpartum weeks, the OC cows' adaptation to the demands of lactation was the worst, they had the most unbalanced metabolism and used more stored lipids compared to T and O cows. This also reflected in higher milk fat percentage and higher ECM production in OC cows during the first six lactation weeks. Fatty acids concentrations in the first week of lactation, related to IR status in the dry period and DMI in the first days of lactation, describe most of the variation in BCS loss during the first six weeks postpartum. Thus, metabolic processes during the dry period and in the first week of lactation are important determinants for metabolic health in the first weeks of lactation.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

HJ, KL, MH, AW, MO – study conception and design;
PK, HJ, KL, MH, AW – acquisition of data;
PK, HJ, KL – analysis and interpretation of data;
PK – drafting of the manuscript;
PK, HJ, KL, MR, MH, AW, MO – critical revision and approval of the final manuscript.

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