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Effects of rumen-protected conjugated linoleic acid (CLA) on performance of primi- and multiparous cows in the transition period

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ABSTRACT. The aim of this study was to determine if the production effects of dietary rumen-protected conjugated linoleic acid (RP-CLA) supplemented to dairy cows during the transition period depend on parity. Twenty four Polish Holstein-Friesian pregnant cows and sixteen pregnant heifers were allocated to four treatments: 1. primiparous cows fed a diet without RP-CLA; 2. primiparous cows fed a diet with RP-CLA (60 g/cow/day); 3. multiparous cows fed a diet without RP-CLA; and 4. multiparous cows fed a diet with RP-CLA (60 g/cow/day). The RP-CLA was offered in *pre-* and *postpartum* total mixed ration diets from day 21 before expected parturition until day 60 after parturition. Dry matter intake, milk yield and fat, and energy corrected milk yield were not affected by RP-CLA supplementation. RP-CLA supplementation reduced milk fat content by 10.9%, but had no effect on either protein or lactose content, or on yield of fat, protein and lactose. Body condition score (BCS), BCS changes and energy balance (EB) were not affected by RP-CLA addition. Plasma insulin-like growth factor 1 concentration increased in multiparous, but decreased in primiparous cows in response to RP-CLA supplementation (parity × diet interaction). The results of this study confirm that RP-CLA supplementation in transition period lowers milk fat content, but has no effect on milk energy output and EB of the cows. Although multiparous cows had a tendency for a greater decrease in milk fat content due to RP-CLA supplementation, an effect of RP-CLA on milk yield, milk composition and EB in primi- and multiparous cows was similar.

Introduction

A reducing effect of dietary rumen-protected conjugated linoleic acid (RP-CLA) supplementation on dairy cow milk fat content is widely known and commonly used to enhance efficiency of milk production (Odens et al., 2007; Hutchinson et al., 2011, 2012; Pappritz et al., 2011; Hötger et al., 2013; Galamb et al., 2017). When RP-CLA is supplemented to the diet, particularly *trans*-10, *cis*-12 isomer, *de novo* fatty acid (FA) synthesis in the mammary gland is reduced (Bauman and Griinari, 2002; Bauman et al., 2008), mostly throughout the inhibition of the mammary expression of genes encoding enzymes involved in milk fat synthesis (Baumgard et al., 2002).

When less milk fat per kg of milk is being synthesized in the mammary gland due to the RP-CLA

supplementation, more glucose may be available for milk synthesis and/or to support the glucose requirements of body tissues (Moore et al., 2004; Hötger et al., 2013). Specifically, spared glucose due to inhibition of fat synthesis in the mammary gland can be directed to peripheral tissues or milk lactose synthesis (Hötger et al., 2013; Benninghoff et al., 2015). Increased milk yield due to RP-CLA supplementation shown in many studies (Odens et al., 2007; van Soosten et al., 2011; Schlegel et al., 2012; Galamb et al., 2017) is most likely due to directing of spared glucose to lactose synthesis in the mammary gland, the major osmoregulator for mammary uptake of water (Rigout et al., 2002).

Since primiparous cows are still growing animals, the use of nutrients for growth seems to be prioritized over their use for milk synthesis (van Knegsel et al., 2007; Wathes et al., 2007). In this context, glucose spared by RP-CLA-induced milk fat content depression in this group of cows might be preferentially used for tissue growth rather than lactose synthesis, as opposed to what is observed in multiparous cows. Consequently, it could be assumed that the effect of RP-CLA supplementation on milk yield is lower in primi- than in multiparous cows. Moreover, if in primiparous cows the RP-CLA supplementation lowers milk fat content and at the same time does not increase milk yield or increases it much less than in multiparous cows, its effect on energy balance (EB) may also differ between parities. RP-CLA supplementation should rather increase EB of primiparous cows but not milk yield. So far, Sippel et al. (2009) have observed no differences in how primi- and multiparous cows responded to RP-CLA supplementation in milk fat content and milk yield. However, the cows used in their study were in their mid-lactation period when negative EB is rarely a problem. There is no information published in literature on whether the effect of RP-CLA supplementation may differ between primi- and multiparous cows in the transition period when negative EB is common (Drackley, 1999).

We hypothesized that although the RP-CLA effect on milk fat synthesis may not differ between primi- and multiparous cows, glucose saved from milk fat synthesis could be used differently in primi- and multiparous cows, which may affect EB and milk yield. The objective of this study was to examine the effects of supplementing dietary RP-CLA during the transition period on milk yield and composition, as well as EB in primi- and multiparous cows. In this study, RP-CLA supplementation was initiated *prepartum* based on evidences that it may positively influence *postpartum* performance (Galamb et al., 2017; Oliveira et al., 2018).

Material and methods

The experimental procedures were approved by the Local Ethics Committee for Experiments on Animals in Kraków (Poland), followed the Polish Law on Animal Protection which in turn is in accordance with EU Directive 2010/63/EU for animal experiments.

Animals and treatments

Twenty four Polish Holstein-Friesian pregnant cows (parity 2.96 ± 0.81; body weight (BW) 696 ± 85 kg) and sixteen pregnant heifers (BW 531 ± 26 kg) were randomly assigned to four treatments according to a two factorial design with the effects of parity and dietary RP-CLA supplementation as the main effects. The treatments were: 1. primiparous cows fed a diet without RP-CLA (PCLA−), n = 8; 2. primiparous cows fed a diet with RP-CLA (PCLA+), n = 8; 3. multiparous cows fed a diet without RP-CLA (MCLA−), n = 13; and 4. multiparous cows fed a diet with RP-CLA (MCLA+), n = 11. Experimental period started on day 21 (± 3.9) before expected parturition and lasted until day 60 *postpartum*. RP-CLA supplementation was initiated 3 weeks before expected calving based on results of other studies showing that CLA supplementation prior to calving increases dry matter (DM) intake (DMI) *prepartum* (e.g., Bernal-Santos et al., 2003; Castañeda-Gutiérrez et al., 2005) and decreases body fat mobilization more effectively than its supplementation only *postpartum* (Galamb et al., 2017). The cows were fed *pre-* and *postpartum* total mixed rations (TMR), which were balanced according to INRA (2007). The diets were fed *ad libitum*, once daily at 7:00, in an amount that assured 10% refusals (as fed) the next day.

The RP-CLA (Lutrell® Pure, BASF SE, Ludwigshafen, Germany) containing silica (about 20%), hardened vegetable fat (originated from sunflower oil; about 47%), and Lutalin (33%; containing about 30% of *cis*-9*, trans*-11 CLA and about 30% of *trans*-10*, cis*-12 CLA; other methylated fatty acids which occur naturally in sunflower oil) was used as a source of CLA. The supplement was a special inert complex of CLA isomers protected by the lipid encapsulation technique. Prior to feeding, every week, RP-CLA was mixed with part of triticale grain in a 1:9 ratio (as fed) to facilitate mixing with TMR. This mixture was offered both *pre-* and *postpartum* to RP-CLA cows and heifers in an amount ensuring provision of 60 g of the RP-CLA/cow/day (approximately 0.6 kg of mixture of RP-CLA and triticale/cow/day; Table 1).

Table 1. Ingredients and chemical composition of *pre-* and *postpartum* diets for cows

Indices	Diets			
	Prepartum		*Postpartum*	
	RP-CLA−	RP-CLA+	RP-CLA−	RP-CLA+
Ingredients, % of DM[1]				
maize silage	45.00	45.00	49.00	49.00
grass silage	11.00	11.00	14.00	14.00
wheat straw	15.00	15.00	3.00	3.00
high moisture maize grain ensiled	9.00	9.00	9.00	9.00
oat grain	1.32	1.32	1.65	1.65
triticale grain[2]	5.28	5.28	6.25	6.25
rapeseed meal	3.60	3.60	4.50	4.50
soybean meal	8.00	8.00	10.00	10.00
sodium bicarbonate	–	–	0.90	0.90
Co-bind A-Z[3]	0.06	0.06	0.07	0.07
mineral-vitamin mixture for dry cows	1.74	1.74	–	–
mineral-vitamin mixture for lactating cows	–	–	1.63	1.63
RP-CLA[4]	–	+	–	+
Nutrient composition, on DM basis				
ash, %	7.0	7.0	7.3	7.3
crude protein, %	12.8	12.8	13.6	13.6
ether extract, %	3.0	2.9	3.5	3.6
aNDF[5], %	39.6	39.6	35.3	35.3
starch, %	25.7	25.7	27.3	27.3
UFL[6], unit/kg	0.88	0.88	0.93	0.93
PDIN[7], g/kg	86.0	86.0	99.0	99.0
PDIE[8], g/kg	89.0	89.0	97.0	97.0

[1] DM – dry matter; [2] including 0.6 kg/cow/day, which was mixed with RP-CLA; [3] Mycotoxin binder (Delacon, Biotechnik GmbH, Steyregg, Austria); [4] RP-CLA – rumen-protected conjugated linoleic acid (CLA) offered at a dose of 0 (−) or 60 g/cow/day (+); [5] aNDF – neutral detergent fibre determined with heat-stable amylase and expressed inclusive of residual ash; [6] UFL – net energy unit for lactation; [7] PDIN – protein truly digested in the intestine, calculated considering N supply to the rumen; [8] PDIE – protein truly digested in the intestine, calculated considering energy supply to the rumen

The daily dose of RP-CLA was evenly mixed in the feed wagon. Assuming 10% of refusals, the predicted intake of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA was equal to, respectively, 5.5 and 5.5 g/cow/day, and these doses were similar to those used in other studies (Hutchinson et al., 2012; Hötger et al., 2013). Cows were housed in a free-stall barn, in four separate pens, two for *pre-* and two for *postpartum* cows, and were milked twice a day at 7:00 and 16:00. Free access to water and salt licks was ensured during the entire study period.

Sample collection and analysis

Individual feed intake was recorded daily, starting from day 21 before expected parturition and lasting until 60 days in milk (DIM), using the Roughage Intake Control feeding system (Fusion Electronics B.V., Houten, Netherlands). Dry matter intake was calculated based on individual feed intake and DM content of TMR and refusals. The samples of TMR for each treatment and refusals for each cow were collected daily and stored at +2 °C, before pooling weekly and kept frozen (−18 °C) for further analyses. To calculate DMI, weekly TMR and refusal samples were dried in a forced-air oven at 50 °C for 48 h. Then, TMR samples were ground (1 mm sieve) and analysed for DM, ash, crude protein and ether extract using standard analytical procedures (procedure no. 934.01, 942.05, 976.05 and 920.39, respectively; AOAC International, 2005). The starch content was determined by an enzymatic method (Faisant et al., 1995). Neutral detergent fibre (aNDF) was determined with heat-stable amylase according to van Soest et al. (1991), using Ankom 220 Fiber Analyzer (Ankom Technology, Macedon, NY, USA) and expressed inclusive of residual ash.

Milk yield was recorded from first day *postpartum* to 60 DIM. Individual milk samples were taken once a week from morning and evening milkings, starting from 5 to 56 DIM, and milk fat, protein and lactose contents as well as somatic cell count (SCC) were determined immediately after sampling using an infrared milk analyser (Milkoscan, Foss Electric, Hillerød, Denmark). Average daily milk composition and SCC were calculated with consideration of milk yield at morning and evening milkings. Fat corrected (4%) milk yield (FCM) was calculated using the formula: FCM (kg/day) = 0.4 × milk yield (kg/day) + 15 × fat yield (kg/day) (NRC, 2001), whereas energy corrected milk yield (ECM) was calculated using the formula: ECM (kg/day) = 0.25 × milk yield (kg/day) + 12.2 × fat yield (kg/day) + 7.7 × protein yield (kg/day) (Stengärde and Pehrson, 2002). Body condition score (BCS) was recorded on day 21 before expected parturition, and then on days 30 and 60 *postpartum*. Cows were scored by the same person using a 5 point scale, with 0.25 increments (Edmonson et al., 1989). The cows were weighed on days 5, 30 and 60 *postpartum*, before the morning feed delivery. Energy balance was calculated according to Hammon et al. (2009), using the following formula: EB = NEL intake − (ECM × 3.14 + 0.293 × kg of $BW^{0.75}$), where NEL was net energy for lactation and BW was body weight of a cow. Energy balance was expressed in MJ/cow/day and was calculated for days 5, 28 and 56 *postpartum*.

Blood samples were taken before feeding from the jugular vein on day 5 before expected parturition, and on days 5, 30 and 60 *postpartum*. For plasma separation, tubes containing heparin (16 IU/ml blood; Sarstedt, Nümbrecht, Germany) were used. After collection, blood samples were immediately put on ice, cooled and centrifuged at 2300 *g* for 10 min. Blood for serum was collected into tubes containing clot activator (Vacuette, Kremsmünster, Austria). Tubes were left at room temperature until clot forming and then centrifuged at 2300 *g* for 10 min. Plasma and serum were stored at −20 °C until further analysis. Plasma glucose, β-hydroxybutyric acid (BHBA), insulin-like growth factor 1 (IGF-1), leptin, as well as serum insulin and non-esterified fatty acids (NEFA) were determined. Glucose, BHBA, NEFA and insulin were determined in blood samples taken on day 5 before expected parturition, and on days 5 and 30 *postpartum*, whereas IGF-1 and leptin were determined in samples taken on day 60 *postpartum*. Plasma glucose and BHBA were determined on an automatic chemical analyser (Hitachi 902; Hitachi, Tokyo, Japan). For plasma glucose determination, the BioSystems (Barcelona, Spain) kit was used, whereas for plasma BHBA determination, the kit was provided by Diagnostic Systems Laboratories (Sinsheim, Germany). Serum NEFA concentration was determined on an automatic analyser (Cobas-Bio; Roche, Basel, Switzerland) using enzymatic methods and Wako Chemicals GmbH reagents (Neuss, Germany). Plasma IGF-1, leptin, and serum insulin were measured by radioimmune assay (RIA) method on a Wizard 1470 Gamma Counter (Perkin Elmer, Waltham, MA, USA). The SM-C RIA CT (DIAsource, Ottignies-Louvain-la-Neuve, Belgium), multi-species (Millipore, St. Charles, MO, USA) and INS-Irma (DIAsource, Ottignies-Louvain-la-Neuve, Belgium) kits were used, respectively, for plasma IGF-1 and leptin, and serum insulin determination.

Statistical analysis

For DMI data, separately for the *pre-* and *postpartum* period, milk yield, FCM, ECM, milk composition and milk component yield, BW and EB, plasma glucose and BHBA, serum NEFA and insulin concentrations and *postpartum* BCS were analysed as repeated measurements using PROC MIXED of the SAS (SAS Institute, 2002). Because milk composition was determined weekly, data on milk yield and also DMI were limited to 56 days of lactation, and prior to analysis reduced to weekly means, which facilitated data analysis and data interpretation. Therefore, data for milk yield and DMI refer to the first 8 weeks of lactation. Data for SCC were log-transformed prior to analysis to achieve normal distribution of the data. The statistical model included fixed effects of the diet (RP-CLA− vs RP-CLA+), parity (primiparous vs multiparous) and time (day or week), and up to three-way interactions between these effects. The week or the day of the study was considered as a repeated measure, and optimal covariance error structure was chosen based on the lowest Bayesian Information Criterion. Data for *prepartum* BCS, BCS change, plasma leptin and IGF-1 concentrations were subjected to a two-way analysis of variance using PROC MIXED of the SAS, with the effect of diet (RP-CLA− vs RP-CLA+), parity (primiparous vs multiparous) and interaction between those two included in the model as fixed effects. Initial BCS was used as a covariate in the analysis for *postpartum* BCS. Data are presented as least squares means and pooled standard errors. The significance was declared at $P < 0.05$, and trends were discussed when $0.05 < P < 0.15$.

Results

Although crude protein content in the *postpartum* diet can be considered low (13.6% in DM; Table 1), particularly for primiparous cows, PDIN (protein truly digested in the intestine, calculated considering N supply to the rumen) and PDIE (protein truly digested in the intestine, calculated considering energy supply to the rumen) concentrations were within the requirements (INRA, 2007).

Parity did not affect DMI *prepartum*, but DMI *postpartum* was greater for multiparous cows ($P < 0.01$; Table 2) and increased faster after calving than in primiparous cows (parity × time interaction, $P < 0.05$). Multiparous cows produced more milk, FCM and ECM in comparison to primiparous cows ($P < 0.01$). There was no parity effect on milk protein concentration, but the milk of multiparous cows contained more fat and less lactose ($P \leq 0.01$). Multiparous cows had a higher milk fat content up to week 5 of lactation; at the end of the study those differences (weeks 6, 7, 8) were no longer observed (parity × time interaction, $P < 0.05$). The daily yield of milk fat, protein and lactose was greater for multiparous cows ($P < 0.01$). Milk of multiparous cows contained more SCC ($P = 0.02$). There was no parity effect on BCS, although multiparous cows tended to lose more BCS from day −21 *prepartum* to day +30 *postpartum*

Table 2. Effects of rumen-protected conjugated linoleic acid (CLA) supplementation and parity on number of days on treatment *prepartum*, *pre-* and *postpartum* dry matter intake (DMI), milk yield and milk composition and body condition score (BCS) of cows

Indices	Primiparous[1]		Multiparous[1]		SEM	Main effects and their interaction, *P*-values		
	PCLA-	PCLA+	MCLA−	MCLA+		diet	parity	diet × parity
Number of cows	8	8	13	11				
Days on treatment *prepartum*	21.3	20.9	20.2	19.3	1.29	0.63	0.31	0.84
DMI, kg/d								
prepartum[2]	12.5	12.1	11.7	13.2	0.53	0.29	0.81	0.10
postpartum[2,3]	15.9	17.1	19.1	20.2	0.84	0.20	<0.01	0.89
Milk yield[2], kg/d	29.8	30.1	33.9	37.3	1.47	0.24	<0.01	0.32
FCM[4], kg/d	29.6	29.8	38.5	38.5	1.58	0.96	<0.01	0.98
ECM[5], kg/d	29.1	29.1	37.0	37.6	1.18	0.86	<0.01	0.87
Protein[6], %	3.11	3.03	3.07	3.07	0.59	0.50	0.99	0.52
Fat[2], %	3.80	3.64	4.65	3.98	0.16	0.02	<0.01	0.12
Lactose[2], %	4.74	4.74	4.59	4.65	0.43	0.50	0.01	0.47
Protein, kg/d	0.95	0.95	1.09	1.19	0.04	0.21	<0.01	0.22
Fat[2], kg/d	1.16	1.15	1.61	1.53	0.08	0.44	<0.01	0.58
Lactose[2], kg/d	1.45	1.49	1.63	1.82	0.06	0.07	<0.01	0.25
SCC[2,7], × 10[3]/ml	273	341	387	501	90	0.83	0.02	0.72
BCS								
prepartum	3.34	3.34	3.50	3.47	0.13	0.93	0.28	0.93
postpartum[8]	2.83	2.77	2.65	2.70	0.09	0.94	0.22	0.56
BCS change								
days from −21 to +30	-0.53	-0.66	-0.85	-0.75	0.11	0.93	0.07	0.32
days from +30 to +60	-0.06	0.06	0.04	0.00	0.06	0.53	0.75	0.21

[1] diets with rumen-protected CLA offered at a dose of 0 (−) or 60 g/cow/day (+) for primiparous (PCLA) or multiparous (MCLA) cows; [2] significant effect of time ($P < 0.05$); [3] significant effect of interaction parity × time ($P < 0.05$); [4] FCM – fat corrected milk = 0.4 × milk yield (kg/day) + 15 × fat yield (kg/day); [5] ECM – energy corrected milk = 0.25 × milk yield (kg/day) + 12.2 × fat yield (kg/day) + 7.7 × protein yield (kg/day); [6] significant interaction of diet × time ($P < 0.05$); [7] SCC – somatic cell count; [8] average BCS of days 30 and d 60 *postpartum*

Table 3. Effects of rumen-protected conjugated linoleic acid (CLA) supplementation and parity on body weight end energy balance of the cows

Indices	Primiparous[1]		Multiparous[1]		SEM	Main effects and their interaction, *P*-values		
	PCLA−	PCLA+	MCLA−	MCLA+		diet	parity	diet × parity
Body weight[2,3], kg								
day[4] +5	524	537	672	724				
day +30	505	500	635	678	20.1	0.28	<0.01	0.32
day +60	506	505	637	668				
Energy balance[2,3], MJ/(cow × day)								
day +5	−54.1	−48.6	−78.0	−82.7				
day +28	−9.6	2.3	−20.9	−11.1	9.36	0.35	0.16	0.71
day +56	−12.7	−0.9	−1.0	6.7				

[1] diets with rumen-protected CLA offered at a dose of 0 (−) or 60 g/cow/day (+) for primiparous (PCLA) or multiparous (MCLA) cows; [2] significant effect of time ($P < 0.05$); [3] significant effect of parity × time ($P < 0.05$); [4] day relative to parturition

($P = 0.07$). There was no parity effect on EB (Table 3), but there was parity × time interaction on EB ($P < 0.05$) with deeper EB in multiparous cows on day +5 *postpartum* compared to primiparous cows. There was no parity effect on serum NEFA and insulin and plasma IGF-1 (Table 4). Multiparous cows had lower plasma glucose ($P = 0.02$) and plasma leptin concentrations (on day 60 of lactation; $P=0.05$), as well as greater plasma BHBA concentration ($P < 0.01$).

Supplementing RP-CLA neither had an effect on DMI *prepartum* nor *postpartum* (Table 2). However, a tendency ($P = 0.10$) for interaction between the main effects on DMI *prepartum* was shown. This tendency was due to greater DMI for MCLA+ cows compared to MCLA− cows.

Supplementing RP-CLA had an effect neither on milk, FCM nor on ECM yields (Table 2). Although RP-CLA supplemented cows produced 1.7 kg more milk, this difference was not significant.

Table 4. Effects of rumen-protected conjugated linoleic acid (CLA) supplementation and parity of cows on selected blood parameters

Indices	Primiparous[1]		Multiparous[1]		SEM	Main effects and their interaction, *P*-values		
	PCLA−	PCLA+	MCLA−	MCLA+		diet	parity	diet × parity
Glucose[2], mg/dl								
day[3] −5	96.5	94.1	87.5	90.6				
day +5	91.1	87.5	81.1	84.0	2.00	0.45	0.02	0.44
day +30	87.5	83.8	86.3	80.6				
BHBA[2, 4], mM/l								
day −5	0.37	0.30	0.41	0.36				
day +5	0.48	0.51	0.88	0.75	0.09	0.90	<0.01	0.52
day +30	0.38	0.49	0.88	0.85				
NEFA[2,5], mM/l								
day −5	0.30	0.37	0.41	0.39				
day +5	1.00	0.86	1.13	1.02	0.08	0.61	0.22	0.55
day +30	0.65	0.74	0.75	0.87				
Insulin[2], µIU/ml								
day −5	13.5	12.5	21.9	14.7				
day +5	12.0	14.4	9.7	9.2	1.57	0.57	0.60	0.65
day +30	8.1	7.9	10.4	11.4				
Leptin[6], ng/ml	135	123	115	117	5.95	0.44	0.05	0.25
IGF-1[6,7], ng/ml	3.20	2.73	2.81	3.63	0.31	0.59	0.42	0.05

[1] diets with rumen-protected CLA offered at a dose of 0 (−) or 60 g/cow/day (+) for primiparous (PCLA) or multiparous (MCLA) cows; [2] significant effect of time ($P < 0.05$); [3] day relative to parturition; [4] BHBA – β-hydroxybutyric acid; [5] NEFA – non-esterified fatty acids; [6] day 60 of lactation; [7] IGF-1 – insulin-like growth factor 1

With no parity × diet interaction, a higher milk yield of RP-CLA supplemented cows was mainly due to a greater milk production of MCLA+ cows. RP-CLA supplementation reduced milk fat content by 10.9% ($P = 0.02$), but had no effect on milk protein and lactose contents or yield of milk fat, protein and lactose, and SCC. The decreasing effect of RP-CLA supplementation on milk fat content tended (diet × parity interaction, $P = 0.12$; Figure 1) to be higher in multiparous cows (14.4%) than in primiparous cows (4.2%). There was also a tendency for a higher decreasing effect of RP-CLA on milk fat yield in multiparous cows (diet × parity interaction, $P = 0.12$; Figure 2). Because a tendency for less apparent impact of RP-CLA on milk fat content in primiparous cows could be a result of differences in RP-CLA intake per unit of BW (due to differences of DMI and BW between primi- and multiparous cows), ratio of DMI per BW was calculated for each animal and additional statistical

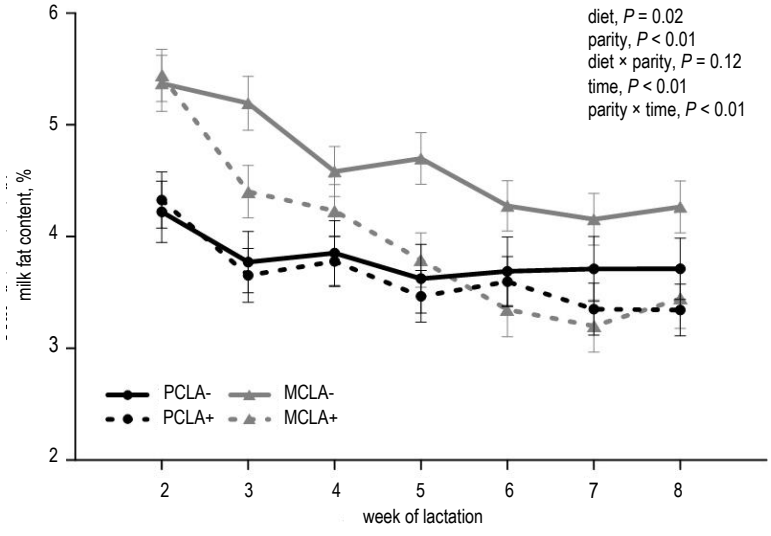

Figure 1. Milk fat content (%) in primiparous (P) or multiparous (M) dairy cows fed diets not supplemented (PCLA− and MCLA−) or supplemented (PCA+ and MCLA+) with 60 g/cow/day of rumen-protected conjugated linoleic acid (CLA)

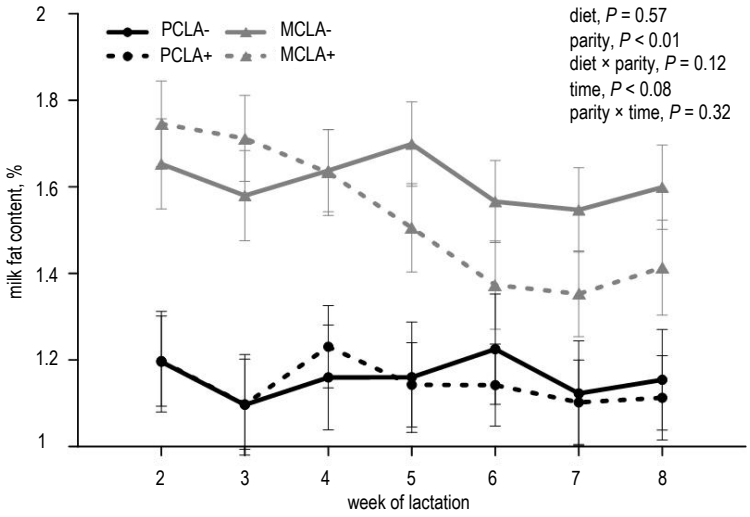

Figure 2. Milk fat yield (kg/day) in primiparous (P) or multiparous (M) dairy cows fed diets not supplemented (PCLA− and MCLA−) or supplemented (PCA+ and MCLA+) with 60 g/cow/day of rumen-protected conjugated linoleic acid (CLA)

analysis was done to test whether DMI/BW differed between treatments and whether this had impact on milk fat content (by including DMI/BW in the statistical model as a covariate). However, neither DMI/BW differed between treatments nor DMI/BW use in the statistical analysis as a covariate affected final results of milk fat content. Irrespective of the parity, milk protein content tended to decrease as lactation progressed, and this effect was more apparent in cows that were fed a diet with RP-CLA (diet × time interaction; $P < 0.05$).

RP-CLA supplementation did not affect BCS and BCS changes during the study. There was neither diet nor diet × parity interaction effects on EB.

Plasma glucose and serum insulin decreased after parturition, whereas plasma BHBA and serum NEFA increased between day −5 and day 5 and then decreased (significant effect of time; $P < 0.05$). RP-CLA supplementation had no effect on the above parameters. RP-CLA supplementation decreased plasma IGF-1 content in primiparous cows but increased plasma IGF-1 in multiparous cows (diet × parity interaction; $P = 0.05$).

Discussion

Low number of replicates, particularly for primiparous cows (n = 8/treatment), is probably the weakest point of this study. However, to the knowledge of the authors, this study is the first comparing a response of primi- and multiparous cows to RP-CLA supplementation in the transition period. So far, an impact of RP-CLA on milk composition and milk yield in primiparous cows has been presented by Sippel et al. (2009); however, in their study, cows were in the mid-lactation period, which makes the results of the present study worth discussing.

Since there are well documented and widely known differences in early lactation between primi- and multiparous cows, which, in most cases, have been confirmed in our study, they will be discussed here only when necessary. Instead, we have placed a greater emphasis on the effects of potential interactions between RP-CLA supplementation and parity.

Although there were some differences among parities in DMI, a lack of differences among parities in DMI per 1 kg of BW let us think that the dose of RP-CLA consumed per kg of BW was the same for primi- and multiparous cows.

The extent of the decrease of milk fat content due to RP-CLA supplementation in the present study is in line with the results of other authors (Baumgard et al., 2002; Pappritz et al., 2011; Hötger et al., 2013; Galamb et al., 2017). Nevertheless, it is worth noting that this decrease was mainly due to milk fat reduction in multiparous cows (diet × parity interaction, $P = 0.12$). This observation, although only numerical, may suggest a greater response of multiparous cows to RP-CLA supplementation, at least in the transition period. Irrespective of the diet, milk fat content in the present study was higher in multi- than in primiparous cows, especially during the first 5 weeks of lactation (data not presented). Higher milk fat content in the first weeks of lactation often indicates mobilization of body fat reserves (Drackley, 1999). This mobilization was likely greater in multiparous cows, as supported

by a tendency for greater BCS loss in this group of animals. Greater mobilization of fat reserves should mean more long-chain fatty acids in milk but less of those which are synthesized *de novo* (Bauman and Griinari, 2003). According to Bauman et al. (2008), RP-CLA containing *trans*-10, *cis*-12 CLA reduces fat content in milk mainly by reducing *de novo* synthesis of FA (C4:0–C14:0, and half of C16:0), but also milk fat synthesis from preformed FA by a reduction of FA uptake from triacylglycerol-rich lipoproteins (by inhibiting lipoprotein lipase). Unfortunately, milk FA composition was not determined in this study. A lack of diet × parity interaction on BCS change let us assume that RP-CLA affected FA composition similarly in both parity groups.

A lack of the effect of RP-CLA supplementation on milk yield corresponds with the results of previous studies conducted on cows in early lactation (Castañeda-Gutiérrez et al., 2005; Hutchinson et al., 2011; Pappritz et al., 2011; Galamb et al., 2017), but differs from the findings of others who observed higher milk yield in RP-CLA supplemented cows (Hutchinson et al., 2012; Schlegel et al., 2012; Hötger et al., 2013). Although milk yield was numerically greater for RP-CLA supplemented cows in the present study (by 1.7 kg/day) and this increase was mainly due to increased milk production in multiparous cows, milk yield was not different between RP-CLA supplemented and not supplemented cows, likely due to the huge variation in milk yield between animals. Nevertheless, fat yield, ECM and FCM were also not affected by RP-CLA supplementation and the effect of its supplementation did not differ between primi- and multiparous cows. Also in other (Odens et al., 2007) but not all (Hutchinson et al., 2012; Schlegel et al., 2012; Hötger et al., 2013) studies, RP-CLA supplementation had no effect on fat and energy output in milk. Results of the current study are in line with those reported by Sippel et al. (2009) who showed no differences in response to RP-CLA supplementation between primi- and multiparous cows in midlactation period in terms of milk yield and fat yield.

Moore et al. (2004) hypothesized that the extra glucose spared by the decrease in milk fat synthesis may be partitioned toward lactose synthesis in the mammary gland, resulting in higher milk production. On the other hand, spared glucose can be also used by peripheral tissues. The latter direction of glucose use seems to be especially justified in primiparous, still growing cows (NRC, 2001). However, considering a lack of significant diet × parity interactions on milk fat content and milk yield in the present study, this hypothesis cannot be proved. In line with lack of differences in milk fat production, as well as FCM and ECM, RP-CLA supplementation in the current study also did not affect energy balance of the cows, both primi- and multiparous, further supporting lack of substantial difference in how primi- and multiparous cows respond to RP-CLA in the transition period. RP-CLA supplementation did not affect blood BHBA and NEFA changes around calving in both parity groups, supporting a lack of its effect on metabolic status irrespective of parity. Although there were tendencies for higher BCS losses in multiparous cows, they were not related to RP-CLA supplementation and they had no effect on neither BHBA nor NEFA content.

According to Hutchinson et al. (2012), in studies where RP-CLA did not improve EB, energy saved from the decrease in milk fat synthesis was likely partitioned toward greater milk production, such that milk energy output remained unchanged. The results of the current study support this hypothesis.

Except for blood IGF-1 at 60 DIM, there was no diet or diet × parity interaction on blood parameters studied and on their changes within the transition period. With the available data, no clear explanation can be given why RP-CLA supplementation decreased plasma IGF-1 concentration in primiparous cows and increased it in multiparous cows (diet × parity interaction; $P = 0.05$). It has been shown that RP-CLA supplementation may increase liver production of IGF-1 and increase its plasma concentration (Castañeda-Gutiérrez et al., 2007; Csillik et al., 2017). The mechanism by which *trans*-10, *cis*-12 CLA increases circulating IGF-1 levels remains unclear, but seems to be independent of EB, and perhaps mediated by changes in hepatic sensitivity to insulin (Castañeda-Gutiérrez et al., 2007). Liver production of IGF-1 is regulated by growth hormone (GH) and the effect of RP-CLA on both GH and IGF-1 may be different in primi- than multiparous cows. This would require further studies.

Conclusions

Results of the current study confirm earlier studies showing that dietary rumen-protected conjugated linoleic acid (RP-CLA) supplementation during the transition period lowered milk fat content, but had no effect on milk energy output and energy balance. Although multiparous cows had a tendency for a greater decrease in milk fat content

due to RP-CLA supplementation, the effect of RP-CLA on milk yield, milk composition and EB in primi- and multiparous cows was similar.

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