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Effect of conjugated linoleic acid supplementation during the transition period on plasma metabolites and productive and reproductive performances in dairy cows

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ABSTRACT

The objective of this study was to determine the effects of dietary conjugated linoleic acid (CLA) supplementation during the transition period on plasma metabolites and productive and reproductive performance in dairy cows. Seventy five multiparous Holstein cows approximately 3 weeks before expected calving date were randomly assigned to 1 of 4 diets containing: (1) CLA21 (n = 18), rumen protected CLA (75 g/h/d, Lutrell Pure, BASF, Ludwigshafen, Germany) from 21 d before parturition (dbp) to 21 d postpartum (dpp); (2) CLA42 (n = 19), rumen protected CLA from 21 dbp to 42 dpp; (3) PO21 (n = 18), palm oil (75 g/h/d) from 21 dbp to 21 dpp; or (4) PO42 (n = 20), palm oil from 21 dbp to 42 dpp. Daily milk yield and body condition score (BCS) at calving and 42 dpp were recorded. Milk fat, protein and lactose were determined weekly. Blood samples were collected from 8 cows in each dietary group at 20.2 ± 3.2 (mean ± SEM), 0, 10, 21 and 42 d relative to calving (day 0) to determine plasma concentration of glucose, cholesterol, triglyceride, high density lipoprotein (HDL), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA) and insulin. Milk yield was greater in cows fed diets supplemented with CLA compared to those fed PO diets, conversely overall milk fat content and fat yield was lower in cows fed CLA compared to those fed diets supplemented with PO. There were no differences among diets for dry matter intake (DMI). Cows in CLA21and those in PO42 dietary treatments had the least and greatest BCS loss, respectively from calving until 42 dpp. Plasma concentrations of glucose and cholesterol were greater in CLA fed cows compared with PO fed cows. Plasma NEFA and BHBA were lower in PO21 and CLA21 cows than in PO42 and CLA42 cows. However, dietary treatment had no effect ($P \geq 0.05$) on plasma concentration of triglyceride, HDL, SGOT, SGPT and insulin.

Abbreviations: CLA, conjugated linoleic acid; NEB, negative energy balance; BCS, body condition score; BW, body weight; TG, triglyceride; HDL, high density lipoprotein; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; NEFA, nonesterified fatty acids; BHBA, β-hydroxybutyrate; TAI, timed artificial insemination.

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In addition, reproductive performance did not differ among dietary groups. In summary, feeding CLA during transition period decreased milk fat percentage, BCS loss and increased plasma concentrations of glucose and cholesterol, and milk yield. Extending supplementation of either CLA or PO from 21 to 42 dpp increased plasma concentrations of BHBA and NEFA, but did not alter milk composition and yield. Reproductive performance of dairy cows did not differ among dietary treatments.

1. Introduction

A sudden increase in nutrient requirements for milk production immediately after calving and a simultaneously decrease in dry mater intake (beginning a few days before calving) are the major challenges of modern dairy cow during the transition period (Drackley, 1999). It has been reported that the nutrient requirements of lactating dairy cows usually exceed what they receive from the diet, resulting in a negative energy balance (NEB), which enhance the risk of decreased production and reproduction performance (Butler, 2003). Increasing energy density of the diet, by adding fat, has been considered as a possible solution to diminish the impact of NEB during transition period (Beam and Butler, 1997). However, fat supplementation might result in reduced dry matter intake or increased milk yield, exacerbating the NEB condition in early postpartum dairy cows (van Knegsel et al., 2007). Nevertheless, it has been shown that dietary fat has positive effects on reproduction regardless of its effects on cow's energy status (Santos et al., 2008).

Conjugated linoleic acid (CLA) is a common name for fatty acids with 18-carbon and a conjugated double bond that have been shown to influence several biological processes (Ryder et al., 2001; Yang and Cook, 2003; Abolghasemi et al., 2016). The two extensively studied isomers of CLA are *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA; the latter has been shown to inhibit lipid synthesis in mice and swine (Park et al., 1997; Thiel-Cooper et al., 2001). In lactating dairy cattle, supplementation of *trans*-10, *cis*-12 CLA isomer also decreased milk fat synthesis in a dose-dependent manner (Bauman et al., 2008). Because approximately 50 percent of the energy required for milk production is used for milk fat synthesis (Tyrrell and Reid. 1965), CLA supplementation has been examined as a tool to reduce NEB and improve milk yield and fertility in dairy cattle (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005, 2007). In this regard, Bernal-Santos et al. (2003) supplemented CLA (30.4 g/d) from 14 d before parturition through 140 d postpartum and found that milk yield, fat content and fatty acid composition were affected after the second week of lactation, but energy balance did not differ between CLA and control cows. Castaneda-Gutierrez et al. (2005) evaluated two doses of rumen-protected CLA (31.6 *vs.* 63.2 g/d) as an approach to reduce milk energy output in transition cows and improve reproductive performance during early lactation. They found that 63.2 g/d of CLA supplementation from 14 d before parturition through 63 d postpartum resulted in 21% decrease milk fat yield, reduced milk energy output, but net energy balance and reproductive performance were not significantly affected. In a follow-up study, Castaneda-Gutierrez et al. (2007) compared 63.0 *vs.* 76.0 g/d of lipid encapsulated CLA mixture from 20 to 56 d postpartum. There was no difference in energy balance but plasma IGF-I was greater and progesterone during the early luteal phase tended to be greater in cows supplemented with 76.0 g/d of CLA. Based on these studies, it would be plausible to explore the effect of a high dose of CLA during transition on plasma metabolites, production and reproduction of dairy cattle. Additionally, it would be interesting to know whether an extended CLA supplementation during the postpartum period would be necessary to achieve positive effects on plasma metabolites, and productive and reproductive performance.

The primary objective of this study was to examine the effects of supplementation with conjugated linoleic acid (CLA, 75 g/h/d) during transition period on plasma metabolites and productive performance in dairy cows. A secondary objective was to determine whether CLA supplementation from 21 d before parturition to 21 or 42 d postpartum would improve reproductive performance of dairy cows.

2. Materials and methods

2.1. Animals and diets

This study was carried out in a large commercial dairy herd in the north of Iran with all animal experimental procedures approved by the Iranian Ministry of Agriculture (experimental permission No. 1028). During the study, the ambient temperature and the relative humidity of the region were 11–27 °C and 52–75%, respectively.

Cows were housed in free-stall barns bedded with sand and equipped with fans. All cows had free access to fresh water during the experimental period. Seventy-five multiparous (3.3 ± 1.7 lactations) Holstein cows approximately 3 weeks before expected calving were randomly assigned to 1 of 4 diets containing: (1) PO21 (n = 18), palm oil (75 g/h/d) from 21 d before parturition (dbp) to 21 d postpartum (dpp); 2) PO42 (n = 20), palm oil from 21 dbp to 42 dpp; 3) CLA21 (n = 18), rumen protected CLA (75 g/h/d, Lutrell Pure, BASF, Ludwigshafen, Germany) from 21 dbp to 21 dpp; or 4) CLA42 (n = 19), rumen protected CLA from 21 dbp to 42 dpp. Diets were isonitrogenous and isoenergetic and offered as TMR (Table 1). The TMR was formulated for a lactating dairy cow of 650 kg body weight (BW), producing 45 kg of milk per day, according to NRC (2001) guidelines. Fat supplements were manually mixed with 425 g of a specially formulated concentrate to ensure palatability

Table 1
Ingredients and nutrient composition (g/kg DM unless otherwise noted) of pre and postpartum diets.

Item	Prepartum	Postpartum
Ingredient (g/kg DM[1])		
Alfalfa hay mid	244	228
Wheat straw	96	–
Corn silage	288	220
Beet pulp, dehydrated	–	41
Corn grain, ground	141	164
Barley grain, rolled	85	145
Soybean meal, 48%	70	145
Soybean whole, roast	–	8
Cottonseed whole	10	–
Wheat	21	–
Sodium bicarbonate	–	9
Salt	–	4
DCP	–	3
Magnesium oxide	–	3
Glucosa	20	13
Choline chloride	8	4
Palm oil[2]/CLA supplement[3]	5	3
Mineral and vitamin premix[4]	12	10
Composition (g/kg DM)		
NEL (Mj/kg DM)	6.7	7.3
Fat	32.7	40.8
Crude protein	133.5	171.0
Neutral detergent fiber	368.0	320.0
Acid detergent fiber	231.0	223.0
Non fibrous carbohydrate	365.3	403.9

[1] Gram per kilogram of dry matter.
[2] Energizer-RP10; Iffco, Johor Bahru Johor, Malaysia.
[3] Lutrell Pure, BASF, Ludwigshafen, Germany.
[4] Contained (per kg): 500,000 IU of Vitamin A, 100,000 IU of Vitamin D, 1000 mg of Vitamin E, 9000 mg of P, 195,000 mg of Ca, 2000 mg of Mn, 55,000 mg of Na, 2000 mg of Zn, 2000 mg of Fe, 280 mg of Cu, 100 mg of Co, 100 mg of Br, 1 mg of Se, and 3000 mg of Anti-oxidant.

Table 2
Fatty acid composition of conjugated linoleic acid (CLA) and palm oil supplements added to the pre and postpartum diets.

Fatty acid (g/kg DM)	CLA	Palm oil
C14:0	2.4	–
C15:0	<1	–
C16:0	111.1	850
C16:1	<1	–
C18:0	533.4	20
C18:1 *cis*-9	104.3	–
C18:2 *cis*-9,*cis*-12	6.7	–
C18:2 *trans*-10,*cis*-12 CLA	92.6	–
C18:2 *cis*-9,*trans*-11 CLA	103	–
C18:3 *cis*-9,cis-12,*cis*-15	<1	–
Others	45.5	130

and were top-dressed once daily at the morning feeding. Fatty acid composition of CLA and palm oil fat supplements is presented in Table 2. Rumen protected CLA provided 7.5 g/h/d of each *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 isomers.

Total mixed rations were sampled every 2 weeks and pooled each month for analysis. Feed samples were dried at 65 °C for 24 h and then ground to a size able to pass through a 1-mm screen (Retsch SM 100; Retsch GmbH, Haan, Germany). Feed samples were analyzed for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber according to the official methods of analysis of AOAC (2000). Values of net energy for lactation were calculated according to nutrient requirements of dairy cattle values (NRC, 2001).

2.2. Dry matter intake, milk production and milk composition

Animals were fed TMR twice a day during prepartum (08:00 and 16:00) and four times a day during postpartum (07:00, 11:00, 15:00 and 23:00) period. Cows were group fed as described by deVries et al. (2005). Briefly, for every feeding time TMR was mixed one hour prior to its delivery to the cows. Dry matter intake for each group for each day was recorded by subtracting DM weight of orts from that of delivered feed.

Cows were milked three times daily at 06.00, 14.00, and 22.00 and milk yield (Kg) was recorded daily up to 42 dpp using electronic milk meters (BouMatic, Madison-Wisconsin, USA). Milk samples were taken weekly (every Monday) from each

milking, and a composite was formed based on proportion of daily yield. Milk composition (fat, protein and lactose) was determined using electronic milk analyzer (Milk Analyzer-jet2, Dairy scan Co. Bulgaria) according to operating instruction provided by manufacturer.

2.3. Milk fatty acid analysis

Milk sample were taken from a subset of 5 animals per group at 3, 21 and 42 d postpartum to determine milk fatty acid. Lipid extraction was performed by a nonsolvent method using centrifugation according to Feng et al. (2004), and methylation was performed using commercial aqueous concentrated HCl as an acid catalyst in methanol/water as described by Ichihara and Fukubayashi (2010).

The fatty acid composition was quantified using gas chromatograph (Yl 6100GC, youngling, South Korea) equipped with capillary column (60 m × 0.25 mm i.d.with 0.25-μm film thickness; BPX70, SGE Analytic Science, Australia). Carrier gas was helium (20 mL/min). A gradient temperature program was used, and total time for each run was 40 min. Injector temperature was 250 °C and detector temperature was 280 °C.

2.4. Body weight and body condition score

All cows were weighted and body condition score (BCS) determined at initiation of the study (20.2 ± 3.2 dfp), the day after parturition, and at 21 and 42 dpp. The same technician assigned BCS to each cow on a scale of 1 (emaciated) to 5 (over conditioned) with 0.25 increments as described by Edmonson et al. (1995).

2.5. Blood sampling

Blood samples were obtained from a subset of 8 cows per group at −20.2 ± 3.2, 0, 10, 21 and 42 d relative to calving (Day 0) to determine blood metabolites. Samples were collected by coccygeal venipuncture into evacuated tubes containing sodium heparin (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA), plasma was harvested within 20 min after collection by centrifugation (1000 × *g* for 5 min, 4° C) and stored at −20° C until analyses.

Plasma concentration of glucose, cholesterol, triglyceride (TG), high density lipoprotein (HDL), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were determined by an automated biochemical analyzer (Technicon RA 1000; Bayer, NY) using commercial kits (Pars Azmoon Co., Tehran, Iran) according to manufacturer's instructions.

Plasma concentration of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) were determined by calorimetric method (Randox Laboratories Ltd., Ardmore, UK) and insulin was determined by the ELISA method using commercial kit (Monobind Inc, Lake Forest, CA, USA) according to manufacturer's instructions. Intra- and inter-assay coefficients of variation for insulin were 6.1 and 4.8%, respectively and for the other metabolites were <5%.

2.6. Reproductive management

Cows were subjected to a Presynch/Ovsynch timed-AI (TAI) program initiated at 30 ± 3 dpp. The presynchronization consists of two injections of PGF2α (estroPLAN, 250 μg cloprostenol sodium; Parnell Technologies Pty. Ltd., Alexandria, Australia) 14 d apart. The first GnRH (100 μg gonadorelin acetate; GONAbreed, Parnell Technologies Pty. Ltd) of Ovsynch was given 12 d after the last PGF2α; at this time cows also received an intravaginal insert containing 1.56 g of progesterone (Cue-Mate; Bioniche Animal Health, Armidale, Australia). Cue-Mate were removed and PGF2α given 7 d later, followed by a second GnRH treatment administered 56 h after PGF2α. All cows were TAI approximately 16 h following second GnRH of Ovsynch. All inseminations were performed by one technician with commercially available frozen-thawed semen.

Pregnancy diagnosis was performed by ultrasonography (Easi-Scan version 3, BCF Technology Ltd, Livingston, Scotland, UK) at 30 d after AI. Pregnancy was characterized by the presence of fluid, an embryo, and a heartbeat (Dirandeh, 2014). Nonpregnant cows received a PGF2α treatment at pregnancy diagnosis and 100 μg of GnRH 56 h later. Cows were TAI 16 h after GnRH. Cows diagnosed pregnant at d 30, were re-examined by rectal palpation at 60 ± 3 d after AI to confirm pregnancy. Pregnancy loss was considered to have occurred when a cow was diagnosed pregnant at 30 d after TAI and not pregnant at 60 d.

2.7. Statistical analyses

All data were analyzed using SAS (Windows; SAS Institute, Cary, NC, USA). All repeated measurements data (dry matter intake, milk yield, milk components, milk fatty acid composition, BCS, BW and blood metabolites) were analyzed using PROC MIXED of SAS (2001) for repeated measures with the following model; To analyze dry matter intake, average DMI of each group for each day was considered as a replicate.

$$Yijk = \mu + \alpha i + \tau j + (\alpha\tau)ij + Bk + eijkl$$

Table 3
Least square means of plasma metabolites, enzymes and insulin concentrations of Holstein cows fed diets supplemented with palm oil (PO) or conjugated linoleic acid (CLA).

Plasma variable[2]	DIETS[1]				SEM	P-value		
	PO21	PO42	CLA21	CLA42		DIET	TIME	DIET × TIME
Glucose (mg/dL)	64.50[a]	64.22[a]	72.91[b]	73.43[b]	3.03	<0.01	0.01	0.02
Cholesterol (mg/dL)	150.42[a]	148.11[a]	188.41[b]	194.11[b]	9.40	<0.01	0.68	0.51
Triglycerides (mg/dL)	18.11	18.13	17.50	17.52	1.26	0.98	0.55	0.73
HDL (mg/dL)	84.70	87.7	81.40	76.21	8.30	0.57	0.42	0.46
SGOT (IU/L)	95.70	95.01	91.90	106.80	10.97	0.56	0.66	0.53
SGPT (IU/L)	21.20	19.71	18.90	20.70	1.75	0.60	0.19	0.29
BHBA (mmol/L)	0.46[a]	0.56[b]	0.46[a]	0.54[b]	0.01	<0.01	0.01	0.71
NEFA (mmol/L)	0.40[a]	0.53[b]	0.38[a]	0.49[c]	0.01	<0.01	0.72	0.42
Insulin (μIU/ml)	0.11	0.14	0.19	0.24	0.05	0.37	0.45	0.66

[a,b,c]Means with a row with different superscripts differ.

[1] PO21: palm oil (75 g/h/d) from 21 dbp to 21 dpp; PO42: palm oil (75 g/h/d) from 21 dbp to 42 dpp; CLA21: rumen protected CLA (75 g/h/d) from 21 dbp to 21 dpp; CLA42: rumen protected CLA (75 g/h/d) from 21 d to 42 dpp.

[2] HDL (high density lipoprotein); SGOT (Serum glutamic-oxaloacetic transaminase); SGPT (Serum glutamic-pyruvic transaminase); BHBA (β-hydroxybutyrate); NEFA (nonesterified fatty acids).

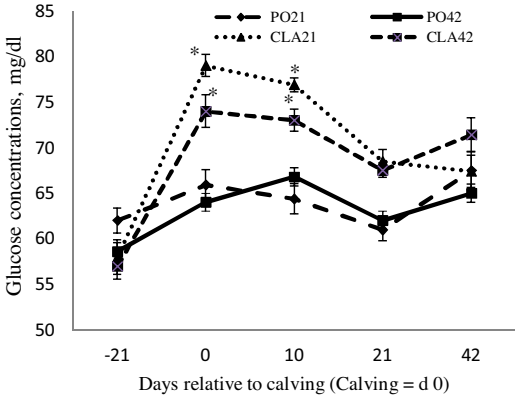

Fig. 1. Plasma glucose concentration in cows fed diets supplemented with palm oil (PO) or rumen protected CLA (CLA) from 21 d before calving to either 21 (PO21, CLA21) or 42 (PO42, CLA42) d postpartum. Cows fed a diet supplemented with CLA (CLA21 and CLA42) had greater ($P < 0.05$) plasma glucose concentrations at 0 (calving) and 10 dpp. (Values are least squares means; n = 8 per group; SEM = 3.03 mg/dL).

where μ is the population mean, αi is the treatment effect, τk is the effect of sampling day or time, (ατ)ij is the interaction effects of treatment and sampling day or time, Bk is random effect of animal, and eijkl is the residual error. Rankit plots and Wilk-Shapiro tests were used to assess the normality of the residuals.

Binomial data (conception rate, pregnancy loss and percentage of cows pregnant at 150 dpp) were analyzed using GLIMMIX procedure of SAS fitting a binary distribution response. Significance and tendencies were declared at $P \leq 0.05$ and $P < 0.10$, respectively, unless otherwise indicated.

3. Results

3.1. Blood metabolites

Mean plasma concentrations of glucose and cholesterol were greater ($P < 0.01$) in CLA fed cows compared with those fed diets supplemented with PO (Table 3). There was a diet by time interaction for glucose; cows fed a diet supplemented with CLA had greater ($P < 0.05$) glucose concentrations at calving and 10 dpp (Fig. 1). Mean plasma concentrations of triglyceride, HDL, SGOT, SGPT and insulin did not differ among dietary treatments ($P > 0.05$). Mean plasma BHBA and NEFA were lower in PO21 and CLA21 cows compared with that in PO42 and CLA42 cows ($P < 0.01$).

3.2. Dry matter intake, milk production and composition, disease incidence

Dry matter intake, milk yield and milk composition data are presented in Table 4. Supplementation with CLA resulted in an increase ($P < 0.01$) of approximately 2 kg/h/d of milk yield (46.64 and 47.68 kg/d in PO21and PO42 *vs.* 48.71 and 49.61 kg/d in CLA21 and CLA42). Milk yield increased over time ($P < 0.001$), whereas no treatment × time interactions was detected. The amount of 3.5% fat corrected milk (3.5% FCM) was lower ($P < 0.01$) in CLA42 cows than PO42 cows (48.81 vs. 51.33 kg/d).

Table 4
Least squares means for DMI, milk yield and milk composition among dietary treatments.

Variable	DIETS[1]				SEM	P-value		
	PO21	PO42	CLA21	CLA42		DIET	TIME	DIET × TIME
DMI (kg/d)	22.24	22.03	22.31	22.09	0.46	<0.31	0.01	0.35
Milk Yield (kg/d)	46.63[a]	47.58[a]	48.73[b]	49.57[b]	0.36	<0.01	0.01	0. 35
3.5% FCM[2] (kg/d)	50.66[ab]	51.33[a]	49.75[ab]	48.81[b]	0.36	<0.01	0.01	0. 35
Milk Fat								
Content (g/kg)	38.30[a]	38.12[a]	34.43[b]	32.25[c]	0.30	<0.01	0.01	0.38
Yield (kg/d)	1.78[a]	1.81[a]	1.66[b]	1.58[b]	0.04	<0.01	0.01	0.11
Milk Protein								
Content (g/kg)	32.64[a]	32.18[ab]	32.04[ab]	31.42[b]	0.11	0.01	0.62	0.37
Yield (kg/d)	1.52	1.53	1.56	1.5	0.02	0.41	0.45	0.42
Milk Lactose								
Content (g/kg)	51.97[a]	50.33[b]	50.24[b]	49.18[b]	0.34	<0.02	0.34	0.28
Yield (kg/d)	2.42	2.40	2.45	2.44	0.03	0.51	0.45	0.19

[a,b,c]Means with a row with different superscripts differ.

[1] PO21: diet supplemented with palm oil (75 g/h/d) from 21 dbp to 21 dpp; PO42: diet supplemented with palm oil (75 g/h/d) from 21 dbp to 42 dpp; CLA21: diet supplemented with rumen protected CLA (75 g/h/d) from 21 dbp to 21 dpp; CLA42: diet supplemented with rumen protected CLA (75 g/h/d) from 21 dbp to 42 dpp.

[2] Fat Corrected Milk = (0.432 × milk yield) + (16.23 × milk fat yield).

Dry matter intake did not differ among dietary groups, but it was increased over time and a treatment × time interactions was not detected.

Milk fat content decreased in CLA fed cows compared to PO fed cows (34.43 and 32.25 g/kg in CLA21 and CLA42 *vs*. 38.30 and 38.12 g/kg in PO21 and PO42, respectively; $P < 0.01$). Furthermore, milk fat yield was greater in PO fed cows (1.76 and 1.79 kg/d in PO21 and PO42 *vs*. 1.64 and 1.56 kg/d in CLA21 and CLA42, respectively; $P < 0.01$). Milk protein ($P = 0.47$) and lactose ($P = 0.47$) composition did not differ among dietary groups (Table 4).

The percentage of metritic cows as indicated with purulent cervical discharge between days 7 to 10 postpartum was the same between CLA and palm oil fed cows (13.2% in CLA cows *vs*. 5.4% in palm oil fed cows; $P = 0.18$). Likewise, the percentage of endometritis was not affected by CLA (36.5% in CLA cows *vs*. 25.8 in palm oil fed cows; $P = 0.40$). The incidence of lameness and mastitis was 8.3, 15, 13.8 and 7.9% in C21, C42, CLA21 and CLA42, respectively ($P = 0.29$).

3.3. Milk fatty acid analysis

There was a reduction in C6:0, C8:0, C10:0 and C16:0 fatty acids in milk fat of CLA fed cows ($P < 0.05$; Table 5). Conjugated linoleic acid fed cows had higher content of C18:1 t fatty acid content in milk fat compared with palm oil fed cows ($P = 0.05$). Milk fat content of *cis*-9, *trans*-11 CLA fatty acid was the same among groups. But *trans*-10, *cis*-12 CLA was higher in milk fat of CLA fed groups while amount of this isomer was highest in milk fat of CLA42 cows ($P < 0.01$).

3.4. Body condition score and body weight changes

Diets had no effects on mean BCS and BW during prepartum period ($P > 0.05$). The mean BCS over the postpartum period was greater in CLA21 cows ($P = 0.02$; Table 6). The BCS loss from calving to 42 dpp was greatest in PO42 and lowest in CLA21 cows ($P < 0.01$). The average BW over the entire study and the change of BW from calving to 42 dpp did not differ among dietary treatments ($P = 0.6$ and $P = 0.73$, respectively).

3.5. Reproductive performance

Conception rate at first and second service and days open did not differ among dietary treatments ($P > 0.05$, Table 7). Albeit not statistical different, pregnancy losses were numerically higher in PO fed cows (33.3 and 28.6% in PO21 and PO42 *vs*. 12.5 and 14.3% in CLA21 and CLA42, respectively, $P = 0.76$). There were no differences in the proportion of pregnant cows at 150 dpp among dietary treatments ($P = 0.68$).

4. Discussion

Dietary supplementation with CLA during transition period resulted in increased plasma glucose concentration in the present study. This is in agreement with previous studies (Odens et al., 2007; Hötger et al., 2013). For instance, Odens et al. (2007) reported an 11% increase in blood glucose concentration in cows supplemented with CLA, which was associated with a possible decrease in insulin sensitivity. Therefore, authors suggested that the increase in blood glucose might be part of the mechanisms by which CLA partitions nutrients from extra mammary tissues toward mammary gland that would

Table 5
Fatty acid composition of milk fat from cows during experimental period.

Fatty acid (g/100 g fat)	DIETS[1]				SEM	P-value		
	PO21	PO42	CLA21	CLA42		DIET	TIME	DIET × TIME
C4:0	1.45	1.51	1.34	1.41	0.14	0.56	0.001	0.12
C6:0	1.12[a]	1.10[a]	0.86[b]	0.80[b]	0.05	0.04	0.01	0. 62
C8:0	0.74[a]	0.75[a]	0.51[b]	0.55[b]	0.04	0.01	0.02	0. 33
C10:0	1.95[a]	1.88[a]	1.43[b]	1.49[b]	0.11	0.01	0.01	0.09
C12:0	2.36	2.27	2.42	2.32	0.21	0.32	0.07	0.46
C14:0	9.2	8.67	8.52	8.25	0.29	0.45	0.03	0.77
C14:1	0.83	0.90	0.76	0.74	0.05	0.37	0.02	0.24
C15:0	1.19	1.09	1.34	1.21	0.06	0.57	0.14	0.18
C16:0	33.08[a]	32.99[a]	30.70[b]	30.71[b]	0.45	0.05	<0.01	0.45
C16:1	3.29	3.26	3.19	3.33	0.18	0.57	0.18	0.21
C17:0	1.11	1.12	1.25	1.27	0.06	0.38	0.01	0.41
C18:0	11.46	11.37	12.16	12.21	0.34	0.72	0.001	0.88
C18:1t	1.39[a]	1.42[a]	1.65[b]	1.74[b]	0.04	0.05	0.11	0.08
C18:2t	0.64	0.62	0.61	0.61	0.06	0.85	0.15	0.24
C18:2c	2.74	2.91	3.06	3.07	0.12	0.43	0.31	0.43
CLAc9t11	0.29	0.27	0.41	0.38	0.06	0.22	0.01	0.45
CLAt10c12	0.01[a]	0.01[a]	0.03[b]	0.04[c]	0.003	<0.01	0.01	0.03
C18:3n3	0.31	0.33	0.45	0.40	0.13	0.38	0.41	0.71
C18:3n6	0.04	0.03	0.03	0.04	0.008	0.58	0.10	0.51
C:20	0.18	0.16	0.18	0.18	0.08	0.82	0.31	0.48
Other FAs	2.27	1.78	2.58	1.75	0.47	0.63	0.01	0.25

[a,b,c]Means with a row with different superscripts differ.

[1] PO21: diet supplemented with palm oil (75 g/h/d) from 21 dbp to 21 dpp; PO42: diet supplemented with palm oil (75 g/h/d) from 21 dbp to 42 dpp; CLA21: diet supplemented with rumen protected CLA (75 g/h/d) from 21 dbp to 21 dpp; CLA42: diet supplemented with rumen protected CLA (75 g/h/d) from 21 dbp to 42 dpp.

Table 6
Least squares means for body condition score (BCS) and body weight (BW) of Holstein cows fed diets supplemented with palm oil (PO) or conjugated linoleic acid (CLA)treatment period.

Variable	DIETS[1]				SEM	P-value		
	PO21	PO42	CLA21	CLA42		DIET	TIME	DIET × TIME
Prepartum period								
BCS (mean)	3.21	3.28	3.18	3.33	0.21	0.46	0.75	0.71
BW (mean, kg)	659	667	660	671	11.28	0.73	0.67	0.58
Postpartum period								
BCS (mean)	2.9[a]	2.9[a]	3.0[b]	2.9[a]	0.03	0.02	0.01	0.63
BCS loss[2]	1.0[a]	1.2[b]	0.7[c]	0.9[a]	0.04	<0.01		
BW (mean, kg)	606	615	599	611	7.50	0.60	0.56	0.71
BW loss[3](kg)	55	57	61	58	3.60	0.73		

[a,b,c]Means with a row with different superscripts differ.

[1] PO21: palm oil (75 g/h/d) from 21 dbp to 21 dpp; PO42: palm oil (75 g/h/d) from 21 dbp to 42 dpp; CLA21: rumen protected CLA (75 g/h/d) from 21 d to 21 dpp; CLA42: rumen protected CLA (75 g/h/d) from 21 dbp to 42 dpp.

[2] Calculated as the difference in BCS from calving to 42 dpp.

[3] Calculated as the difference in BW from calving to 42 dpp.

Table 7
Reproductive performance of Holstein cows fed diets supplemented with palm oil (PO) or rumen protected conjugated linoleic acid (CLA).

Variable	DIETS[1]				SEM	*P*-value
	PO21 (n = 18)	PO42 (n = 20)	CLA21 (n = 18)	CLA42 (n = 19)		
1 st service conception rate, %	29.4	35.0	22.2	26.3		0.85
2nd service conception rate, %	8.3	7.7	28.6	14.2		0.41
Cumulative 1 st & 2nd service conception rate[2], %	35.3	40.0	44.4	36.8		0.95
Days open[3], d	91	72	80	96	12.50	0.55
Pregnancy loss[4], %	33.3	28.6	12.5	14.3		0.76
Cows pregnant at 150 dpp, %	35.2	30.0	44.4	47.6		0.68

[1] PO21: palm oil (75 g/h/d) from 21 dbp to 21 dpp; PO42: palm oil (75 g/h/d) from 21 dbp to 42 dpp; CLA21: rumen protected CLA (75 g/h/d) from 21 dbp to 21 dpp; CLA42: rumen protected CLA (75 g/h/d) from 21 dbp to 42 dpp.

[2] Calculated as percentage of all pregnancies obtained from first and second inseminations to number of cows in each treatment.

[3] Cows were censored if not pregnant at 150 dpp.

[4] Pregnancy losses were considered to have occurred when a cow was diagnosed pregnant at 32 d after timed artificial insemination (TAI) and not pregnant at 60 d after TAI.

increase milk yield. However, the effect of CLA supplementation on plasma glucose concentration, observed in the present study, was not associated to changes in plasma insulin concentration, hence, we hypothesize that it was related to a more efficient use of metabolizable energy (von Soosten et al., 2012; Hötger et al., 2013). In a previous study that evaluated the effect of CLA supplementation on glucose metabolism in early lactation dairy cows, Hötger et al. (2013) observed a decrease in endogenous glucose production with increase plasma glucose concentration as a result of a more efficient use of metabolizable energy. This intriguing finding could be due to an inhibitory effect of CLA on the synthesis of milk fatty acids which may be more important than its positive effect on milk production. A similar improvement in metabolizable energy utilization has been reported by von Soosten et al. (2012), who found more protein accretion and less heat production in CLA fed cows. In contrast, others have reported that CLA supplementation during transition periods did not affect plasma glucose concentration (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005; Medeiros et al., 2010).

Based on our best knowledge, the current study and that by Esposito et al. (2013) are the only reports of an increase in plasma cholesterol concentration in CLA fed cows; all previous studies reported no effects of CLA supplementation on circulating cholesterol concentration (Medeiros et al., 2010; Schlegel et al., 2012; Hötger et al., 2013). In this regard, Schlegel et al. (2012) examined the hepatic expression of several genes involved in various pathways of lipid metabolism and concluded that CLA supplementation had no effect on cholesterol metabolism in liver which, in term, resulted in no changes on cholesterol concentrations in blood. However, a positive correlation between blood cholesterol concentration and milk production was reported in dairy cows (Jóźwik et al., 2012). In the present study, cows fed a diet supplemented with CLA also had higher milk yield. Therefore, the higher blood cholesterol concentration of CLA fed cows may have been due to alterations in the partitioning of metabolites as a result of more milk production rather than alterations in the hepatic cholesterol metabolism. Further studies are needed to elucidate this hypothesis.

In the current study, overall milk fat content and yield was decreased significantly in CLA fed cows compared to PO fed cows. Dietary supplementation with CLA caused 6.8 and 12.8% reduction in milk fat yield and 10.2 and 16.0% reduction in milk fat content in CLA21 and CLA42 cows compared to PO21 and PO42 cows, respectively. The overall milk fat reduction in CLA fed cows was comparable with that reported by Bernal-Santos et al. (2003) and Moore et al. (2004) where CLA supplementation resulted in 12–13% reduction in milk fat content and yield, but lower than the 27% reduction in milk fat yield reported by others (Selberg et al., 2004; Castaneda-Gutierrez et al., 2005; Odens et al., 2007). Differences in the amount of CLA supplemented among studies may be the reason for the discrepancy on the impact of milk fat yield reduction.

Cows fed diets supplemented with CLA yield on average 2 kg of milk more than those fed diets supplemented with PO which is in agreement with other reports (Bernal-Santos et al., 2003; Odens et al., 2007; Medeiros et al., 2010). However, it is in contrast to the reports of Selberg et al. (2004) and Castaneda-Gutierrez et al. (2005) where diets supplemented with CLA during the early postpartum period had no effect on milk yield. Milk fat contributes to more than 50% of the energy in milk (Tyrrell and Reid, 1965); therefore, it is expected that the energy saved, due to milk fat depression in CLA fed cows, should be available for milk and milk component synthesis. In addition, an overall greater BCS loss in PO fed cows compared to that in CLA fed cows may be indicative of a less severe NEB condition in the latter group that can be related to the positive effect of CLA supplementation on milk yield.

Supplementation of CLA during transition period has had controversial effects on blood NEFA and BHBA concentrations. Moore et al. (2004) and Hötger et al. (2013) reported no effects, while others reported a decrease (Odens et al., 2007) or increase (Selberg et al., 2004; Medeiros et al., 2010). In our study, BHBA and NEFA was not affected by CLA supplementation but extending the period of supplementation from 21 to 42 dpp resulted in increased average plasma BHBA and NEFA concentrations regardless of the type of fat supplemented. In cows in the PO42 dietary group, the elevation in plasma concentration of BHBA and NEFA was associated with a greater BCS loss from calving to 42 dpp. However, the same trend regarding BCS was not observed in cows fed diets supplemented with CLA until 42 dpp. In support to our observation, the mobilization of body fat mass for the first 42 dpp was 24.1 kg in cows fed a diet supplemented with stearic acid compared to 14.3 kg in cows fed a diet supplemented with CLA (von Soosten et al., 2012). In the current study, the overall BCS loss was less severe in CLA supplemented cows. Therefore, the changes in plasma BHBA and NEFA concentrations did not reflect the changes in body fat mobilization in cows receiving a diet supplemented with CLA.

Lack of dietary effects on DMI is in agreement with previous TMR-based (Bernal-Santos et al., 2003; Moore et al., 2004; Selberg et al., 2004; Odens et al., 2007) and pasture-based (Mackle et al., 2003; Kay et al., 2006) studies that investigated the effect of CLA supplementation on DMI.

At this study CLA supplementation of transition dairy cows resulted in a reduction in some short- and medium-chain fatty acids and palmitic acid (C16:0) in milk fat of CLA fed cows. It was in agreement with other studies and consistent with the proposed CLA action on *de novo* fatty acid synthesis in mammary epithelial cells (Mackle et al., 2003; Bauman et al., 2008; Medeiros et al., 2010). Fatty acids in milk fats may be originated by *de novo* synthesis in mammary gland (<C16) or uptake of performed fatty acids (>C16) (Neville and Picciano, 1997). C16 fatty acids can originate from both sources (Bauman and Griinari, 2003). The results reported here showed that the CLA mixture used at this experiment provided a high enough dose of *trans*-10, *cis*-12 CLA to decrease the expression of genes involved with *de novo* fatty acid synthesis (Baumgard et al., 2002). The more percentage of *trans*-10, *cis*-12 CLA in milk fat of CLA fed cows at this study is an indication of transfer of this isomer from rumen protected CLA supplement to milk (Castaneda-Gutierrez et al., 2005). Extending supplementation period of CLA resulted in more transfer of *trans*-10, *cis*-12 CLA to milk fat of CLA42 cows.

Fat supplementation can enhance reproductive performance in dairy cows by increasing the size of the preovulatory follicle that results in increased estradiol production (Beam and Butler, 1997; Dirandeh et al., 2013a,b; Badiei et al., 2014;

Jahani-moghadam et al., 2015), improving fertilization rates and embryo development (Cerri et al., 2009), affecting endometrial PGF2α production (Dirandeh et al., 2013a), and improving embryo survival (Mattos et al., 2000; Dirandeh et al., 2013b). Supplementation with CLA had no significant effect on the reproductive performance in this study; however, the findings should be interpreted with caution because the present study was not adequately powered to test binomial variables such as conception rate and pregnancy loss. Albeit not significant, the numerical improvements in the overall conception rate and embryo survival in cows fed a diet supplemented with CLA are comparable to other studies with similar numbers of cows (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005; Medeiros et al., 2010).

5. Conclusions

Supplementation with rumen-protected CLA to dairy cows during the transition period increased plasma concentrations of glucose and cholesterol, and milk yield and decreased milk fat percentage and BCS loss. Extending fat supplementation from 21 to 42 dpp increased plasma concentrations of BHBA and NEFA, but did not alter milk composition and yield. Despite the metabolites and productive responses to CLA supplementation, reproductive performance of dairy cows did not differ among dietary treatments.

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