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Conjugated linoleic acid (CLA) supplementation effects on performance, metabolic parameters and reproductive traits in lactating Holstein dairy cows

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Abstract

The objective of the study was to determine the effects of conjugated linoleic acid (CLA) supplement on milk yield and composition, blood metabolites and reproductive parameters in lactating Holstein dairy cows. Twenty Holstein dairy cows were randomly assigned to one of two dietary treatments: 1) supplementing 110 g per day of fat (control), 2) supplementing 120 g per day of rumen-protected CLA. The diets were formulated to be nutritionally isocaloric and isonitrogenous. The experimental period started 21 days pre-calving and continued until 60 days in milk (DIM). Treatments had no effect on dry matter intake (DMI), body weight (BW) and body condition score (BCS). The CLA treatment increased milk yield (3.04 kg per day and milk lactose concentration, but decreased milk fat concentration and, short and medium chain fatty acids concentrations. No treatment differences were observed in milk protein concentration, milk energy output and net energy balance. Serum concentrations of glucose, cholesterol, triglyceride (TG), insulin, insulin-like growth factor-1(IGF-1), estradiol and progesterone were higher in CLA treated cows when compared to cows fed on the control diet. Serum beta-hydroxybutyric acid (BHBA) concentration was reduced in cows fed on the CLA treatment. Days to first insemination and days open were not different between the two treatment groups. Cows fed on the CLA supplement had increased conception rate from the first service. The results indicated that cows fed on diets supplemented with CLA produced milk with decreased milk fat concentration whereas some related cow blood serum metabolic parameters associated with reproductive response were increased and resulted in an increased conception rate from the first service.

Introduction

Energy requirements for dairy cows is increased during parturition and the onset of lactation. However, dry matter intake (DMI) gradually is decreased over the last three weeks of gestation before calving which exposes cows to a negative energy balance (NEB).[1,2] Consequently, body fat reserves are mobilized to meet energy needs which causes increased serum levels of non-esterified fatty acids (NEFA) and BHBA, leading to metabolic disorders and decreased postpartum reproductive performance.[3]

Furthermore, in cows experiencing NEB, other essential factors ensuring successful reproductive performance such as blood concentration of glucose, insulin and IGF-1 are reduced.[4] There are some approaches to alleviate NEB, such as increasing dietary energy density by increasing the concentrate to forage ratio or dietary fat supplementation. Although these strategies may increase dietary energy concentration, they may also cause decreased DMI leading to an unchanged energy consumption.[5]

Another method to alleviate NEB is reducing milk energy output. Among milk components, milk fat synthesis requires more energy when compared to other components (50.00% of total of milk energy).[6] The trans-10 and cis-12 CLA is an isomer of CLA that reduces milk fat synthesis in the mammary gland.[7] Milk fat synthesis requires glucose and, reduced milk fat synthesis likely uses less glucose, resulting in greater plasma glucose levels.[8] Glucose has been reported to be an important metabolic substrate for the reproductive process and has been shown to be an important energy source for ovarian

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activity.[9] In addition, increased glucose serum concentration has been reported to enhance blood insulin concentration,[4] and increased IGF-1 plasma concentrations and is positively associated with circulating glucose and insulin concentrations.[10] Furthermore, increased insulin and IGF-1 serum concentrations results in gonadotropin secretion from the hypothalamus (GnRH secretion) then gonadotropins such as (LH and FSH) promote growth, follicular and luteal cell proliferation and development, and consequently, induce progesterone (P4) and estradiol (E2) synthesis.[11]

Only a few studies have reported the effects of dietary supplemented CLA on dairy cow performance at different physiological stages. Thus, the objective of this study was to determine dietary CLA supplementation effects on performance, energy balance, blood metabolites and reproductive traits in lactating Holstein dairy cows during the transition and early lactation periods.

Materials and Methods

Animals, treatments and sampling. Twenty lactating Holstein dairy cows were used in the current study. All cows were randomly allocated to one of two dietary treatments with 10 cows in each group in a completely randomized design. The treatments were: 1) 110 g per day of fat supplement containing 65.00% of a Ca-salt mixture of unsaturated fatty acids (UFA, control; Persia Fat Inc., Qom, Iran) and 2) 120 g per day of a mixture of rumen-protected CLA isomer (Lutrell Pure; BASF SE, Ludwig-shafen, Germany). The purity of cis-9, trans-11 and trans-10, cis-12 isomers in the CLA supplement was10.00%. Thus, the CLA supplement provided 12.00 g of each isomer per cow per day. The diets were formulated to meet or exceed NRC[12] requirements and were isocaloric and isonitrogenous (Table 1). The experimental period lasted from 21 days before expected calving until 60 days in milk. Animals were housed in groups of ten per pen. All cows had free access to fresh water during the experimental period. Cows were fed total mix ration (TMR) twice a day during the pre-partum period (8:00 and 16:00) and three times a day during the postpartum (6:30, 14:30 and 22:30) period. The body condition score (BCS) and body weight (BW) for each cow were recorded weekly throughout the experiment. The BCS was assigned based on a 5-point scale by two individuals, and the average of these two scores was used as the assigned value.[13] After parturition, animals were milked three times daily (6:00, 14:00 and 22:00) and milk yield was recorded daily. In addition, during the eight weeks following parturition, milk samples were collected and a mix of all three milkings weekly and milk composition were analyzed immediately using Milkoscan (Funke Geber; LactoStar, West Yorkshire, UK).

Milk fatty acid analysis profile. Milk samples were collected from all animals at the end of the experiment and immediately frozen at – 20.00 ˚C until analysis. The methyl ester forms of the fatty acids were obtain following procedures from Savage *et al.*[14] Milk fat was first extracted from samples, then the fat was converted to the methyl ester form. The methyl ester form of fatty acids was measured using a gas chromatograph (GC-mass 2001; Agilent Technologies, Palo Alto, USA).

Blood metabolites and hormones. Blood samples were taken from each cow prior to the start of the experiment (21 days before expected calving) and collected weekly until 56 days milking. Blood samples were obtained via coccygeal venipuncture into vacuum tubes without any anticoagulant factor. Blood samples were centrifuged immediately at 3,000 rpm for 15 minute and serum was harvested, then stored at – 20.00 ˚C. Cholesterol, glucose, non-esterified fatty acids (NEFA), BHBA, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and blood urea nitrogen (BUN) serum concentrations were analyzed following enzymatic colorimetric methods using an auto analyzer (Alcyon 300; Abbott, New York, USA) with appropriate enzymatic kits including NEFA, BHBA kits supplied by Randox Laboratories Ltd., (Crumlin, UK), cholesterol, glucose, TG, LDL, HDL and BUN kits supplied by Pars Azmoon Laboratories Ltd. (Tehran, Iran). Reproductive hormones such as progesterone (P4), estradiol (E2), insulin and IGF-1 were determined with a radioimmunoassay (RIA) method using ELISA (Boston Therapeutics Inc., Lawrence, USA) with appropriate kits (progesterone and estradiol kit supplied by Monobind, Inc. (Lake Forest, USA), insulin kit supplied by Sigma Diagnostic, Inc. (Livonia, USA), and Bovine IGF-1 Elisa kit supplied by Cusabio Technology LLC, (Wuhan, China). Other reproductive variables such as estrus were detected by visual observation. Cows were then inseminated and days open and conception rate from the first service were determined on each estrus day.

Statistical Analyses. Data from this experiment were analyzed using SAS software (version 9.1; SAS Institute, Cary, USA). The milk production, milk composition, dry matter intake (DMI), BW, BCS, energy balance and blood factors (metabolites and hormones) recorded at different times were analyzed using mixed model methods (Proc MIXED procedure of SAS using repeated measures). The model used in this study contained fixed effects of treatment, time and treatment × time interaction. Animal within treatment was designated as a random variable. The Tukey-Krumer test was used for comparison of means between treatments. For testing differences in milk fatty acid profile, days to first insemination and days open between the two treatments, the SAS *t*-test procedure was used. Conception rate to first service was analyzed using the SAS FREQ procedure with a chi-squared test. The number of services for all cows was analyzed with the SAS logistic procedure using Proc LOGISTIC.

Table 1. Ingredient and chemical composition of the experimental diets during pre- and post-calving periods.

Composition	Pre-partum[1]		Post-partum[2]	
	CLA	Control	CLA	Control
Ingredient (% of Dry matter)				
Alfalfa hay	7.38	7.38	21.97	21.97
Corn silage, normal	33.65	33.65	18.21	18.21
Wheat straw	22.79	22.79	3.77	3.77
Beet sugar pulp, dried	-	-	4.51	4.51
Wheat bran	3.61	3.61	2.00	2.00
Soybean meal	5.25	5.25	8.14	8.14
Whole cotton seeds	-	-	3.36	3.36
Corn grain, ground	6.42	6.42	12.39	12.39
Barley grain, ground	5.59	5.59	12.71	12.71
Soybean whole extrude	2.21	2.21	2.74	2.74
Canola meal	5.01	5.01	3.55	3.55
Meat meal	2.16	2.16	3.93	3.93
Fat supplement[4]	-	0.82	-	0.40
CLA supplement[5]	0.89	-	0.44	-
Di-Calcium phosphate	0.92	0.92	0.46	0.46
Calcium carbonate	0.77	0.77	0.38	0.38
Magnesium oxide	0.23	0.23	0.11	0.11
Salt	-	-	0.19	0.19
Sodium bicarbonate	-	-	0.57	0.57
Vitamin premix[3]	0.61	0.61	0.57	0.57
Ammonium sulfate	1.53	1.53	-	-
Crude protein	14.30	14.30	16.00	16.00
Rumen undegradable protein	4.10	4.10	5.70	5.70
Neutral detergent fiber	40.90	40.90	30.60	30.60
Acid detergent fiber	29.10	29.10	23.20	23.20
Ether extract	3.90	3.90	4.20	4.20
Net energy for lactation (Mcal kg^{-1} of dry matter)	1.53	1.53	1.55	1.55
Calcium	1.20	1.20	1.20	1.20
Phosphorus	0.60	0.60	0.60	0.60

[1] From three weeks pre-calving until parturition; [2] From calving until week eight of lactation; [3] 1.00 kg of the mix contained 60.00 g of Na, 190 g of Ca, 80.00 gr of P, 21.00 g of Mg, 3.00 g of Zn, 2.00 g of Mn, 300 mg of Cu, 100 mg of Co, 100 mg of I, 35.00 mg of Se, 500,000 IU of vitamin A, 200,000 IU of vitamin D, and 4,000 IU of vitamin E; [4] Rumen protected fat powder (Persia Fat); [5]utrell Pure (BASF SE, Ludwigshafen, Germany).

Results

Milk yield (3.04 kg per day, $p < 0.001$), milk protein ($p < 0.006$) and milk lactose ($p < 0.048$) were increased in cows fed on the treatment diet containing CLA (Table 2). Conversely, milk fat concentration and yield were decreased ($p < 0.001$, $p < 0.05$, respectively) in cows fed on CLA. Inclusion of CLA in the diet had no effect on the concentration of milk protein. Also, no differences in BW, DMI, BCS, milk energy output and net energy balance (NEB) were observed between the experimental diets (Table 2).

In this study, dietary supplementing CLA resulted milk fatty acid composition changes (Table 3). Under this condition, milk fatty acid synthesis was decreased, resulting in a reduction in short- and medium-chain fatty acids (< 16 carbons; all $p < 0.05$). However, the proportion of 16:0, 16:1 and other fatty acids containing more than 16 carbons such as 18 carbons and 20 carbons in milk fat were not affected by CLA supplementation. Conversely, CLA supplementation increased the amount of cis-9, trans-11 and trans-10 and cis-12 in milk fat ($p < 0.03$).

Serum metabolites and hormone levels in cows fed on the trial diets during the transition period and early lactation are presented in Table 4. The diets had no effect on mean serum metabolites and hormones levels during the pre-partum period ($p > 0.05$). However, during the post-partum period the consumption of CLA resulted in significantly increased serum concentrations of cholesterol and TG (cholesterol, $p < 0.01$; triglyceride, $p < 0.01$). The mean values of serum BUN, HDL and LDL concentrations were similar between experimental diets. Serum BHBA concentration was decreased ($p < 0.032$) in animals fed on the CLA supplemented diet, whereas serum glucose concentration was increased ($p < 0.01$). Serum NEFA concentrations were similar among treatments.

In the CLA supplemented cows, serum progesterone, estradiol and insulin concentration were increased (progesterone $p < 0.0046$), (estradiol $p < 0.026$), (insulin $p < 0.05$). Cows consuming the treatment diet supplemented with CLA showed greater serum IGF-1 concentrations ($p < 0.09$).

Table 2. Effect of supplementation of CLA on performance and energy variables in transition (over 60-day postpartum period) and early lactation (over 21 day pre-calving to 60 day post-calving) dairy cows.

Item	Treatments*		SEM	*p*-value		
	CLA	Control		Treatments	Time	Treatment × Time
Milk yield (kg per day)	41.20	38.16	0.59	0.001	0.001	0.009
Milk fat (%)	3.33	3.95	0.07	0.001	0.001	0.40
Milk fat yield (kg per day)	1.34	1.48	0.02	0.05	0.001	0.43
Milk protein (%)	3.27	3.17	0.03	0.27	0.001	0.01
Milk protein yield (kg per day)	1.32	1.20	0.01	0.006	0.001	0.09
Milk lactose (%)	4.65	4.40	0.03	0.048	0.01	0.31
Milk lactose yield (kg per day)	1.89	1.68	0.02	0.002	0.001	0.06
Prepartum performance						
Body weight (kg)	697.27	711.40	6.82	0.68	0.52	0.29
Body condition score	3.38	3.28	0.01	0.09	0.001	0.95
Dry matter intake (kg per day)	13.70	13.60	0.03	0.15	0.001	0.59
Postpartum performance						
Body weight (kg)	611.50	613.75	1.21	0.50	0.001	0.27
Body condition score	2.82	2.70	0.01	0.09	0.001	0.71
Dry matter intake (kg per day)	21.30	20.73	0.14	0.65	0.001	0.40
Energy partitioning						
Milk energy output (Mcal per day) †	27.13	26.71	0.36	0.59	0.001	0.55
Net energy balance (Mcal per day) ‡	−3.64	−3.05	0.20	0.40	0.042	0.35

* Cows (n= 10) received 110 g per day fat supplement consisting 65.00% of Ca- salts of unsaturated fatty acids (control), 120 g per day of rumen-protected CLA isomers (CLA group).

† Calculated weekly with use of the NRC[12] equations: (0.0929 × milk fat percentage) + (0.0547 × milk protein percentage) + (0.0395 × milk lactose percentage)] × milk yield.

‡ Calculated weekly with use of the NRC[12] equations, of the NRC[12]: [(DMI × net energy for lactation (NE_L) in the diet) - ($BW^{0.75}$ × 0.08)] + milk energy output.

Table 3. Effect of CLA supplementation on milk fatty acid composition and reproductive variables of dairy cows. Data are presented as average of samples obtained at the end of the week of the experimental period.

Fatty acid (%)	Treatments*		*p*-value
	CLA	Control	
4:0	2.60	3.06	0.06
6:0	1.10	1.80	0.01
8:0	0.74	1.10	0.009
10:0	2.72	4.22	0.001
12:0	3.30	4.80	0.09
14:0	7.00	8.50	0.03
14:1	0.79	0.88	0.97
15:0	0.77	0.83	0.001
16:0	26.80	25.60	0.45
16:1 cis-9	2.01	2.02	0.28
17:0	1.10	1.00	0.20
18:0	12.15	12.78	0.90
18:1 cis-9	23.40	24.80	0.98
Other 18:1	4.40	4.30	0.26
18:2 cis-9, cis-12	4.10	3.80	0.30
Other 18:2	0.35	0.30	0.25
18:2 cis-9, trans-11	0.72	0.46	0.001
18:2 trans-10, cis-12	0.02	0.004	0.03
18:3 cis-9, cis-12, cis-15	0.80	0.72	0.70
20:0	0.40	0.37	0.56
Reproductive variables			
Days to first insemination (day)	78.90 (± 2.10)	82.70 (± 1.85)	0.70
Calving to conception interval (day)	96.60 (± 5.80)	112.30 (± 7.20)	0.54
Conception rate to first insemination (%)	60.00	40.00	0.045
Number of service for all cows	1.50	1.80	0.35

* Cows (n = 10) received 110 g per day fat supplement consisting 65.00% of Ca- salts of unsaturated fatty acids (control), 120 g per day of rumen-protected CLA isomers (CLA group) from 1 to 60 day postpartum.

Table 4. Effect of CLA on blood metabolites and hormones in transition and early lactation dairy cows. Data are presented as average over 21 day pre-calving to 60 day post-calving.

Item	Treatments*		SEM	*p*-value		
	CLA	Control		Treatments	Time	Treatments × Time
Pre-partum						
Cholesterol (mg dL^{-1})	115.27	115.07	3.31	0.98	0.36	0.46
Triglyceride (mg dL^{-1})	19.58	17.54	0.71	0.58	0.53	0.26
Blood urea nitrogen (mg dL^{-1})	12.47	12.33	0.44	0.03	0.14	0.08
High density lipoprotein (mg dL^{-1})	27.10	26.13	1.30	0.87	0.06	0.40
Low density lipoprotein (mg dL^{-1})	74.00	73.50	3.20	0.99	0.065	0.83
Beta-hydroxybutyric acid (mmol L^{-1})	0.46	0.48	0.02	0.50	0.42	0.70
Glucose (mg dL^{-1})	59.20	58.60	0.52	0.60	0.01	0.78
Non-esterified fatty acids (mmol L^{-1})	0.12	0.13	0.02	0.22	0.27	0.23
Progesterone (ng mL^{-1})	8.40	7.60	0.38	0.23	0.12	0.61
Estradiol (pg mL^{-1})	121.10	120.20	3.94	0.84	0.24	0.99
Insulin (µIU mL^{-1})	2.40	2.20	0.19	0.64	0.89	0.88
IGF-1 (ng mL^{-1})	110.10	105.50	4.33	0.67	0.11	0.91
Post-partum						
Cholesterol (mg dL^{-1})	190.30	145.70	6.80	0.03	0.01	0.02
Triglyceride (mg dL^{-1})	18.10	15.54	0.44	0.001	0.63	0.18
Blood urea nitrogen (mg dL^{-1})	11.90	12.60	0.30	0.47	0.04	0.08
High density lipoprotein (mg dL^{-1})	47.90	46.10	1.69	0.26	0.001	0.90
Low density lipoprotein (mg dL^{-1})	128.20	121.50	4.26	0.10	0.001	0.90
Beta-hydroxybutyric acid (mmol L^{-1})	0.45	0.55	0.02	0.02	0.05	0.13
Glucose (mg dL^{-1})	53.20	48.60	0.32	0.01	0.01	0.86
Non-esterified fatty acids (mmol L^{-1})	0.38	0.41	0.04	0.52	0.50	0.50
Progesterone (ng mL^{-1})	3.68	2.87	0.34	0.03	0.01	0.34
Estradiol (pg mL^{-1})	94.10	86.20	1.72	0.08	0.004	0.98
Insulin (µIU mL^{-1})	2.50	2.10	0.07	0.02	0.006	0.90
IGF-1 (ng mL^{-1})	111.10	103.50	1.94	0.13	0.01	0.91

* Cows (n = 10) received 120 g per day fat supplement consisting 65.00% of Ca- salts of unsaturated fatty acids (control), 120 g per day of rumen-protected CLA isomers (CLA group).

There was no difference (Table 3) among days to first service (78.30-day vs 82.70 day for CLA treatment and control, respectively). In addition, no days open differences were observed between the two treatments. Numbers of service per conception or AI/conception tended to be lower in the CLA supplemented group than the control group. The interval from calving to conception was decreased when cows were supplemented with CLA ($p < 0.045$).

Discussion

During the transition and early lactation periods, dairy cows are typically unable to consume adequate DMI to avoid a negative energy balance. Unbalanced energy is reported to increase metabolic diseases such as ketosis and fatty liver, and decrease lactation and reproductive performance by Ospina *et al.*[3] In the present study, supplementing 12.00 g per day of CLA isomers (cis-9, trans-11 and trans-10, cis-12 CLA) produced different effects from previous reported findings.[15-17]

In the present study, feeding dietary CLA decreased milk fat concentration and milk fat yield. Baumgard *et al*. suggested that of all CLA isomers, trans-10 and cis-12 caused the inhibition of fatty acid synthesis through a reduction in the expression of genes encoding many lipogenic enzymes, such as acetyl-CoA carboxylase, fatty acid synthase, and fatty acid desaturase.[7] Similar findings were reported in previous studies.[16,18,19]

Feeding cow diets supplemented with CLA resulted in increased serum glucose concentration. In ruminant animals, acetate and butyrate are the main substrates for *de novo* FA synthesis.[6] When FA synthesis in ruminant animals is decreased, the glucose use is decreased, resulting in an increased serum glucose. The present results were in agreement with reports from Rezaei Roodbari *et al.,*[18] Odens *et al.*[20] and Hötger *et al*.[21] but in contrast with studies by Csillik *et al.*[17] and Hutchinson *et al.*[22]

In the current study, cows fed on supplemental CLA had increased milk and milk protein yields. The increase in milk production and milk protein yield was probably associated with the extra energy which was resulted from the decrease in milk fat synthesis, impacting/affecting the overall energy expenditure in milk production and milk protein synthesis.[22] An increase in milk production is supported by previous studies Galamb *et al.*[16] and Rezaei Roodbari *et al.*[18] Although in studies conducted by Odens *et al.*[20] on CLA supplementation did not affect milk yield.

Milk lactose concentration and yield were also increased in cows fed on diets supplemented with CLA. The present study suggested that increased lactose yield could be due to high milk yield from cows fed on the dietary treatments that included CLA supplementation. This was supported by Hötger *et al*. who hypothesized that lactose synthesis was increased in cows fed on dietary CLA due to a high availability of glucose for lactose synthesis.[21]

Milk energy output and energy balance were not affected by CLA supplementation. Bernal-Santos *et al.,*[15] Hötger *et al.,*[21] and Castañteda-Gutiérrez *et al.*[23] observed similar results. Nevertheless, in studies conducted by Odens *et al.*[20] and Hutchinson *et al.,*[22] energy balance was improved with CLA supplementation. Bernal-Santos *et al.*[15] hypothesized that energy savings resulting from decreased milk fat synthesis tended to increase milk production, resulting in similar milk energy output and energy balance. In addition, for the reasons mentioned above, the serum NEFA concentrations were not different between diets in the present study.

Decreased BHBA serum concentration was observed from cows fed on the CLA treated diets compared to the control diet. We attributed the BHBA concentration decrease to high glucose and insulin concentrations because these cows consumed similar quantities of feed throughout the experimental period. Similar results were reported in studies by Galamb *et al.,*[16] Rezaei Roodbari *et al.*[18] and Captui Olivera *et al.*[19]

The CLA treatment had no effect on the cows' BW and BCS when compared to cows fed on the control diet, because the energy saved by the cows fed on the supplemental CLA treatment was spent for milk production and any residual energy was used to improve BCS. This finding was in agreement with Sheikh *et al.*[24]

In the current study, CLA supplementation caused increased blood serum cholesterol and TG concentrations. The mechanism by which CLA supplementation increased cholesterol concentration in blood serum was unclear. Rezaei Roodbari *et al.* suggested that higher blood cholesterol concentration might be due to changes in metabolite partitioning from more milk production rather than changes in hepatic cholesterol metabolism.[18] This result were in agreement with reports by Rezaei Roodbari *et al.,*[18] Hötger *et al.,*[21] and Esposito *et al.*[25]

Dietary CLA increased serum insulin concentration. Grummer *et al.*[4] reported that when serum glucose concentration increases, serum insulin concentration may also increase. This finding was in agreement with Csillik *et al.*[8] and Csillik *et al.*[17]

Serum IGF-1 concentration was elevated from cows fed on the CLA supplemented diet. The liver is the main source for IGF-1 production. Rutter *et al.* observed that an increase in plasma IGF-1 concentration is positively associated with circulating glucose and insulin concentrations.[10] Butler *et al.* showed that when dairy cows in early lactation go to negative energy balance, the liver is refractory to growth hormone (GH), resulting in decreased circulating IGF-I concentrations.[26] But, increased insulin serum concentration restores the coupling of the GH-GHR-IGF-I axis, which increases circulating IGF-I concentrations. This was consistent with our observations.

The serum P4 and E2 concentration from cows fed on supplemental dieatary CLA was increased. The high level of P4 and E2 in the postpartum period might be related to the high serum IGF-1 and insulin concentrations. IGF-1 and insulin cause increase in growth, proliferation and development of follicles and the corpus luteum (CL), along with P4 production and binding sites of LH on the theca cells Perks *et al.*[27] Thus, Csillik *et al.* observed that CLA supplementation increased IGF-1 production in the liver.[8] Additional cholesterol is used for P4 synthesis in the CL.[5]

The conception rate from the first insemination was increased in cows fed on the supplemental dietary CLA treatment. The present study suggested that improvement in conception rate to first service was related to trans-10, cis-12 CLA isomers levels in the diet, blood metabolite concentrations (glucose and cholesterol) and blood hormone levels (IGF-1, insulin, estradiol and progesterone) and might be independent of energy balance. Progesterone is one of several important factors necessary for successful conception because P4 prepares the uterus for embryo implantation. Furthermore, P4 is necessary for maintaining pregnancy, thus, when P4 serum level is high the first service pregnancy rate is increased.[5]

Additionally, Wathes *et al.* hypothesized that increased blood glucose concentration might facilitate uterine involution and prepare the uterus for pregnancy establishment and maintenance.[28] Moreover, Green *et al.* found that conception rate from first service is high in animals having elevated serum glucose concentrations during the first 30 day postpartum.[29]

Supplementing CLA to the diet of dairy cows had no effect on negative energy balance or serum NEFA concentration. Supplementing the cow's diet with CLA, however, decreased milk fat synthesis in the mammary gland resulting in high blood serum glucose levels, thus, enhancing serum insulin and IGF-1 concentrations. In addition, high IGF-1 and insulin levels may cause a stimulation of growth, development and proliferation of granulosa cells and CL, associated with increased P4 and E2 production. Ultimately, the results of this study indicated that for a successful reproductive performance, it is necessary to consider factors responsible for optimizing P4 and E2 concentrations.

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Conflicts of interest

The authors declare no conflicts of interest.

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