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Influence of a supplement containing conjugated linoleic acid on dairy performance, milk fatty acid composition, and adipose tissue reactivity to lipolytic challenge in mid-lactation goats

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ABSTRACT

The objective of this work was to evaluate the effect of the supplementation of conjugated linoleic acid (CLA; 4.5 g of *cis*-9,*trans*-11 C18:2 and 4.5 g of *trans*-10,*cis*-12 C18:2) on milk performance, milk fatty acid (FA) composition, and adipose tissue reactivity in dairy goats fed a high-concentrate diet based on corn silage. Twenty-four multiparous dairy goats in early to mid lactation were used in a 10-wk trial, with a 3-wk adaptation to the experimental total mixed ration that contained corn silage (35%, dry matter basis), beet pulp (20%), barley (15%), and a commercial concentrate (30%). Goats were randomly allocated to 2 experimental groups and they were fed 45 g/d of a lipid supplement (either CLA or Ca salts of palm oil added on top of the total mixed ration). Individual milk production and composition were recorded weekly, and milk FA composition was analyzed in wk 2, 5, and 6. In the last week of the trial, an isoproterenol challenge was performed for 12 goats before morning feeding. The CLA supplementation had no effect on dry matter intake (DMI), body weight (BW), milk yield, milk protein content, and lactose yield and content, but it significantly decreased milk fat yield and content by 18 and 15%, respectively. The decrease in milk fat yield was related to a lower secretion of FA synthesized *de novo*, of the medium-chain FA, and to a lesser extent of the long-chain FA that are taken up from the peripheral circulation. The CLA supplementation decreased the proportion of the sum of C16:0 and C16:1 and the sum of total *cis* C18:1, and it increased the proportions of the sum of long-chain (C >16) and the sum of *iso* FA without modification of the total *trans* C18:1 and the sum of FA synthesized *de novo* (C <16). During the first 25 min relative to isoproterenol injection, the maximal concentrations, the increases above basal concentration, the changes in area under the curve, and the total area under the curve

for glucose and nonesterified FA were not affected by CLA treatment. In conclusion, CLA supplementation associated with a high-concentrate diet based on corn silage resulted in decreased milk fat yield, increased net energy balance, and it did not affect the sensitivity of the adipose tissue to lipolytic challenge in lactating goats.

Key words: conjugated linoleic acid, milk fatty acid composition, lipolytic challenge, goat

INTRODUCTION

Conjugated linoleic acids (CLA) are geometric and isomeric isomers of linoleic acid that are produced during ruminal biohydrogenation of linoleic acid (Loureço et al., 2010). Conjugated linoleic acids are mainly produced when high levels of PUFA and high percentages of rapidly fermentable carbohydrates are associated (Bauman et al., 2003). Among the CLA isomers, the *trans*-10,*cis*-12 C18:2 isomer is known to be the most potent isomer for decreasing milk fat content (MFC) in dairy cows (Baumgard et al., 2000), even if *cis*-10,*trans*-12 and *trans*-9,*cis*-11 isomers induce similar responses (Sæbø et al., 2005; Perfield et al., 2007). Molecular-based approaches indicated that *trans*-10,*cis*-12 C18:2 simultaneously downregulated the abundance of transcript of key genes involved in *de novo* synthesis of FA, uptake of long-chain FA, and desaturation of FA in the mammary gland of cows (Shingfield et al., 2010; Bauman et al., 2011). In dairy goats, feeding ruminally protected *trans*-10,*cis*-12 C18:2 resulted in a smaller decrease in MFC than in cows (Lock et al., 2008; Shingfield et al., 2009), reflecting a lower sensitivity of the goat mammary gland to the antilipogenic effect of *trans*-10,*cis*-12 C18:2.

It has been shown that the stage of lactation, the duration of the trial, and the form of protection of dietary *trans*-10,*cis*-12 C18:2 affect the response of mammary gland to CLA supplementation (Bauman et al., 2008) but less attention has been paid to diet composition. In cows (de Veth et al., 2006) and in ewes (Weerasinghe et al., 2012), CLA may alter partitioning

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of nutrients to support an increase in milk and protein yield when dietary energy is limited. Moreover, feeding CLA to dairy cows fed a high-concentrate diet (high starch-low NDF) resulted in a smaller decrease in MFC than in cows fed a low-concentrate diet (low starch-high NDF), suggesting that dietary ingredients or diet composition (or both) may affect the response or sensitivity of the mammary gland to *trans*-10,*cis*-12 C18:2 (Glasser et al., 2010). In dairy goats, reduced MFC was observed with a low-concentrate-low-starch diet based on alfalfa hay (Shingfield et al., 2009), and with a high-concentrate diet based on hay (Lock et al., 2008) during short-term trials. To date, no information is available on the response to *trans*-10,*cis*-12 C18:2 of dairy goats fed high-concentrate diets based on corn silage during a long-term trial.

In opposition to changes observed in the mammary gland, expression of lipogenic enzymes and of genes related to lipid synthesis in subcutaneous adipose tissue were increased during short-term *trans*-10,*cis*-12 C18:2 supplementation in cows (Harvatine et al., 2009), reflecting the partitioning of spared energy away from milk toward adipose tissue. Simultaneously, von Soosten et al. (2011) reported reduced mobilization of retroperitoneal adipose tissue in early lactating cows fed CLA, which may suggest reduced lipolysis in adipose tissue. Accordingly, changes in plasmatic NEFA after a β -adrenergic challenge (epinephrine) in cows fed *trans*-10,*cis*-12 C18:2 were small (Baumgard et al., 2002; de Veth et al., 2006). However, *trans*-10,*cis*-12 C18:2 decreased adipocyte size in primiparous cows during early lactation, suggesting that CLA may stimulate adipose tissue lipolysis (Akter et al., 2011). Adipose tissue response to adrenergic challenge in cow is altered according to changes in body energy input and output (McNamara, 1988), which are affected by CLA. In dairy goats, no study has reported on the effect of *trans*-10,*cis*-12 C18:2 on reactivity of adipose tissue to lipolytic signals.

In consequence, this trial studied the long-term effect of *trans*-10,*cis*-12 C18:2 supplementation on milk performance, milk FA composition, energy metabolism, and reactivity of adipose tissue evaluated by a β -agonist challenge in early to mid-lactating goats fed a high-concentrate diet based on corn silage.

MATERIALS AND METHODS

Animals and Diets

Twenty-four multiparous dairy Saanen or Alpine goats (BW = 63.7 ± 8.0 kg, 30 ± 4 DIM; mean \pm SD) were used for 10 wk, with the first 3 wk for adaptation to experimental diets. Twelve of the goats were fitted

with a ruminal cannula and a T-type duodenal cannula for determination of the diet digestibility and measurement of duodenal flow (not described here) and they were housed in metabolism crates after the adaptation period. The 12 other goats were kept in individual pens. At the initiation of the trial, the average milk yield (mean \pm SD) was 3.9 ± 1.3 kg/d, with a milk fat content (mean \pm SD) of 35.0 ± 5 g/L and a milk protein content (mean \pm SD) of 32 ± 2 g/L.

The goats were allocated to 2 groups according to their milk production and fat content measured 30 d after parturition. From parturition to the start of the trial (wk 0), all goats were fed ad libitum twice per day (0800 and 1700 h) a TMR consisting (on a DM basis) of a mixture of grass hay (20%), dehydrated alfalfa pellets (20%), beet pulp (30%), and a commercial concentrate (30%; Fluvia Selectunic, Sanders, France). The dietary transition was made in the first week of the adaptation period for corn silage, followed by the addition of lipid supplements in the second week. Throughout the experimental period of 7 wk (wk 3 to 9), the goats were fed ad libitum a TMR containing corn silage (35% on a DM basis), beet pulp (20%), barley (15%), and the same commercial concentrate (30%). The CP, OM, NDF, ADF, and starch contents (DM basis) of the basal diet without adding either Ca salts of palm oil (control, CTL) or CLA supplement were 118, 922, 381, 188, and 175 g/kg of DM, respectively. The NE_L of the TMR was 1,500 kcal/kg of DM. The composition of FA of the basal diet is given in Table 1. Lipid supplements (CTL supplement vs. CLA, 45 g/d) were top-dressed on the TMR and thoroughly hand mixed. The CTL supplement consisted of Ca salts of palm oil (Saphir Intense; Eolia, Bruz, France). The CLA supplement (Lutrell; BASF Animal Nutrition, Château-Gontier, France) had a lipid content of 65% and a FA composition of 15% *trans*-10,*cis*-12 C18:2, 15% *cis*-9,*trans*-11 C18:2, 9% 16:0; 42% 18:0, 12% *cis*-9 C18:1, and 1% *cis*-9,*cis*-12 C18:2, corresponding to a daily distribution of 4.5 g of *cis*-9,*trans*-11 C18:2/d and 4.5 g of *trans*-10,*cis*-12 C18:2/d. The CTL supplement had a lipid content of 90% with 50% C16:0; 35% of C18:1 *cis*-9, and 10% C18:2 *cis*-9, *cis*-12. Lipid supplements distribution was 15 g and 30 g in the morning and afternoon feed allocations, respectively.

Experimental Measurements and Sampling

From wk 2 onwards, feed intake was recorded daily. Three diet samples were collected at the end of the adaptation period (wk 2), in the middle (wk 6), and in wk 9 for feed composition analyses and total lipid determination. Goats were weighed once per week at the same time in the middle of the day. During the

trial (from wk 0 to 9), 2 milk samples of 30 mL (one at the evening milking and one at the following morning milking) were individually collected each week with a preservative (Bronopol; Grosseron SA, Saint-Herblain, France) and stored at 4°C until milk composition analysis (protein, fat, and lactose content). During wk 1, 5, and 6, 2 additional milk samples of 30 mL (one at the evening milking and one at the following morning milking) were individually collected without preservative, and stored at -18°C for milk FA composition.

Isoproterenol Challenges

In the last week of the trial, an intravenous isoproterenol (**ISOP**) challenge was performed on the 12 cannulated goats fed the CTL or CLA diets. A solution of ISOP (Sigma-Aldrich, Saint-Quentin-Fallavier, France) of 40 µg/mL was prepared in NaCl (9‰) the day before the challenge as described by Gagliostro and Chilliard (1991) and was kept at 4°C. The day of challenge, the solution was heated on a hot plate agitator (37°C), and a dose of 4 nM ISO/kg of BW (1 µg/kg of BW) was injected within 15 s into the left jugular vein of each goat immediately after morning milking. During the challenge, the 12 goats were not fed. Blood samples were collected from the jugular vein into heparin vials at -20, -5, +5, +10, +15, +20, +25, +30, +45, and +60 min relative to the injection. Blood samples were immediately centrifuged at $1,780 \times g$ for 10 min at 4°C and plasma was stored at -18°C until analysis for glucose, NEFA, BHBA, and insulin.

Laboratory Analysis

For each time point, 1 feed sample was dried in a forced-air oven at 90°C for 24 h to determine DM content and then ground and stored at room temperature until determination of the OM, NDF, ADF (Van Soest et al., 1991), and starch (Glucosquant glucose/HK; Roche/Hitachi Diagnostics, Mannheim, Germany) content. Dry matter, ash, and enzymatic starch in feed were determined following International Organization for Standardization (**ISO**) standards: ISO (1999; 6496), ISO (2002; 5984), and ISO (2004; 15914), respectively. The 2 other feed samples were immediately stored at -20°C and later lyophilized and ground for total lipid determination and FA analysis according to Folch et al., (1957).

Methyl esters from milk or feed samples were injected with an autosampler into a Varian CP 3800 gas chromatograph equipped with a flame ionization detector (Varian SA, Les Ulis, France) on a CP-Sil 88 (100-m \times 0.25-mm i.d. fused silica capillary column; Interchim, Montluçon, France). The carrier gas was ultra-pure

Table 1. Fatty acid profile and contents of the basal diet

Item	Basal diet
FA profile, % of total FA	
C14:0	2.23
C16:0	23.34
C18:0	3.79
<i>cis</i> -9 C16:1	0.49
<i>cis</i> -9 C18:1	27.41
<i>cis</i> -9, <i>cis</i> -12 C18:2	32.48
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	3.33
FA, mg/g of DM	
C14:0	0.031
C16:0	0.324
C18:0	0.053
<i>cis</i> -9 C16:1	0.007
<i>cis</i> -9 C18:1	0.381
<i>cis</i> -9, <i>cis</i> -12 C18:2	0.451
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.046
Total FA	1.29

helium with a flow of 1 mL/min. Injector and detector temperatures were maintained at 250°C. The split ratio in the injector was 20:1. The initial oven temperature was 50°C (for 1 min) and was then increased at 10°C/min to 170°C (held for 78 min) and then increased again at 5°C/min to 220°C (held for 15 min). Individual FA were identified by comparison of retention time to those of pure standards (Supelco Inc., Bellefonte, PA).

For plasma glucose, NEFA, and BHBA analyses, an autoanalyzer (Cobas-Mira Roche/Hitachi, Mannheim, Germany) was used with kits (Glucosquant glucose/HK, Roche/Hitachi Diagnostics; NEFA-HR(2), Wako Chemicals GmbH, Neuss, Germany). β -Hydroxybutyrate was analyzed by the method of Barnouin et al. (1986). Insulin was analyzed with an ELISA kit (10-1202-01; Mercodia AB, Uppsala, Sweden).

Statistical Analysis

Milk yield; fat, protein, and lactose yields and contents; DMI; and BW measured during the trial were evaluated using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) for repeated measures according to the following statistical model:

$$Y_{ijkl} = \mu + A_i + B_j + AB_{ij} + C_k + D_l + Cov_l + \varepsilon_{ijkl}$$

where Y_{ijkl} is the response variable, μ is the overall mean, A_i is the fixed effect of treatment (CTL vs. CLA), B_j is the fixed effect of time (7 wk), AB_{ij} is the interaction between the treatment and time, C_k is the cannula effect (cannulated vs. intact), Cov_l is a covariate measured at the end of wk 2, and ε_{ijkl} is the residual error. Goat was considered as a random effect (D_l). A first-order autoregressive [AR (1)] covariance structure was used for repeated measures. The same model was

Table 2. Live BW, DMI, net energy balance, and milk production and composition in lactating goats fed 45 g/d of Ca salts of palm oil (control, CTL) or 45 g/d of conjugated linoleic acid (CLA; mixture of 2 isomers: *trans*-11,*cis*-9 C18:2 and *trans*-10,*cis*-12 C18:2)¹

Item	Treatment			P-value		
	CTL (n = 12)	CLA (n = 12)	SEM	CLA	Time (T)	CLA × T
Live BW, kg	60.2	61.8	1.714	0.51	0.0004	0.89
DMI, kg/d	3.04	3.01	0.096	0.81	<0.0001	0.71
Milk production, kg/d	3.82	3.74	0.14	0.18	<0.0001	0.91
Milk yield, g/d						
Fat	175.8	144.7	7.5	<0.0001	<0.0001	0.80
Protein	122.3	117.1	4.11	0.14	<0.0001	0.95
Lactose	190.6	188.2	6.93	0.52	0.76	0.76
Milk composition, g/L						
Fat	45.8	38.9	1.37	<0.0001	<0.0001	0.82
Protein	32.1	31.6	0.53	0.16	0.28	0.60
Lactose	48.6	48.8	0.34	0.44	0.80	0.91
Energy balance, ² kcal/d	-82	177	97.89	0.02	0.14	0.88

¹Values represent least squares means, with data obtained during wk 2 as covariates.

²Net energy balance during the trial = (net energy intake + net energy value of the CLA or Ca salt of palm oil) - (NE_M + NE_L).

used to evaluate the effect of CLA, time, and CLA × time interaction on each milk FA measured during wk 5 and 6, with wk 2 values used as a covariate. Preliminary statistical analysis showed that the cannula effect and CLA × cannula interaction effect were not significant; therefore, these effects were withdrawn from the model.

Treatment effects on plasma concentrations of NEFA, glucose, insulin, and BHBA, after ISOP injection were tested for baseline concentrations (baseline values calculated as the average at -20 and -5 min before ISOP injection), maximal concentrations (at 5 or 10 min after injection), maximal increase above basal value, changes in the area under the curve (AUC; calculated as the positive incremental area that was above baseline value during the first 25 min relative to ISOP injection), and total AUC (total AUC, calculated as the area from -20 into 25 min after injection), as described by Cardoso et al. (2011). These data were analyzed using the GLM procedure of SAS (SAS Institute Inc.) according to the following model:

$$Y_{ij} = \mu + A_i + \varepsilon_{ij},$$

where Y_{ij} is the response variable, μ is the overall mean, A_i is the fixed effect of treatment (CTL vs. CLA), and ε_{ij} is the random residual error. Least squares means are reported and treatment effects are declared significant at $P < 0.05$.

RESULTS

Animal Performance

The BW, DMI, milk yield, and milk protein and lactose yields and contents were unaffected by CLA (Table

2). Compared with the CTL, the CLA treatment significantly decreased milk fat yield and content by 18 and 15%, respectively. The CLA treatment increased the net energy balance (NEB) by 260 Kcal/d. No interactions were observed between CLA treatment and time for BW, DMI, NEB, milk yield, or milk protein and lactose yields and contents, or milk fat yield and milk fat content.

Milk FA Profile

The *trans*-10,*cis*-12 C18:2 isomer was detected only in the milk of goats fed the CLA treatment, with no change in the proportion of the *cis*-9,*trans*-11 C18:2 isomer between the groups. Apparent efficiency of transfer of *trans*-10,*cis*-12 C18:2 from diet to milk $\{100 \times [\textit{trans}\text{-}10,\textit{cis}\text{-}12 \text{ C18:2 output in milk (g/d)}] / [\textit{trans}\text{-}10,\textit{cis}\text{-}12 \text{ C18:2 intake (g/d)}]\}$ was 1.9%. The interaction between the CLA treatment and time was not significant for most of milk FA. Compared with the CTL, the CLA treatment did not affect the proportions of the sums of SFA, PUFA, or FA with C <16 in milk fat. It increased the sum of ISOP FA (Table 3). When the FA were considered individually (Appendix Table A1), the CLA treatment decreased numerically or significantly the proportions of the linear FA from C8 to C13, and C16:0 in milk fat. It increased the proportions of C4:0, C14:0, C18:0, C19:0, C20:0, and C22:0. The CLA treatment did not affect the sum of *trans* C18:1 (Table 3), but it tended to increase the proportion of the *trans*-11, *trans*-13 + *trans*-14, and *trans*-16 isomers of C18:1. Compared with CTL, the CLA treatment reduced the sum of *cis* C18:1 isomers, *cis*-9 C18:1, and the sum of C16:0 + *cis*-7 C16:1. It also reduced the ratios of *cis*-5 C14:1/C14:0, *cis*-9 C18:1/C18:0, and *cis*-9,*trans*-11

Table 3. Summation of some groups of milk FA (g/100 g of milk fat) in lactating goats fed 45 g/d of Ca salts of palm oil (control, CTL) or 45 g/d of conjugated linoleic acid (CLA; mixture of 2 isomers: *trans*-11,*cis*-9 C18:2 and *trans*-10,*cis*-12 C18:2)¹

Item	Treatment			P-value		
	CTL (n = 12)	CLA (n = 12)	SEM	CLA	Time (T)	CLA × T
ΣSFA ²	72.49	72.56	0.41	0.41	0.82	0.04
Σ <i>iso</i> ³	0.97	1.06	0.04	0.01	<0.0001	0.17
Σ <i>cis</i> C18:1	13.44	11.79	0.51	0.001	0.55	0.61
Σ <i>trans</i> C18:1	5.62	6.23	0.46	0.32	0.31	0.08
ΣMUFA ⁴	19.3	20.1	0.61	0.23	0.38	0.06
ΣPUFA ⁵	4.32	4.32	0.12	0.98	0.78	0.06
Σ C <16	38.13	37.30	0.54	0.29	0.37	0.08
ΣC16:0 + C16:1 ⁶	28.99	27.56	0.57	0.05	0.0002	0.45
Σ C >16	31.48	33.74	0.59	0.02	0.46	0.22
Desaturation ratio						
<i>cis</i> -5 C14:1/C14:0	0.012	0.008	0.008	0.0001	0.99	0.98
<i>cis</i> -9 C18:1/C18:0	1.96	1.42	0.51	<0.0001	0.45	0.57
<i>cis</i> -9, <i>trans</i> -11 C18:2/ <i>trans</i> -11 C18:1	0.38	0.30	0.02	0.02	0.60	0.22

¹Values presented are least squares means, with data measured during wk 2 as covariates. Full details of milk FA composition are given in the Appendix.

²Saturated FA including pair and odd FA.

³Includes *iso* C14:0, *iso* C15:0, *iso* C16:0, and *iso* C17:0.

⁴Monounsaturated FA = Σ (*cis*-5 C14:1, *cis*-6 C15:1, *cis*-7 C16:1, total *cis* C18:1, total *trans* C18:1, and *cis*-11 C20:1).

⁵Polyunsaturated FA = Σ (all isomers of C18:2, *cis*-9,*cis*-12,*cis*-15 C18:3, *cis*-6,*cis*-9,*cis*-12 C18:3, and C20:3n-3).

⁶Includes *anteiso* C17:0.

C18:2/*trans*-11 C18:1 by 33.3% 27.6, and 21.1% respectively. The CLA treatment increased the sum of >C16 FA by 7.2% (Table 3).

The CLA treatment significantly reduced milk secretion of <C16 FA synthesized de novo (353 vs. 290 mmol/d for CTL and CLA, respectively) and the sum of C16:0 + *cis*-7 C16:1 (194 vs. 154 mmol/d), and it

tended to decrease ($P = 0.07$) the secretion of >C16 FA in milk (184 vs. 164 mmol/d; Figure 1).

Response of Plasma Concentrations to an Isoproterenol Challenge

Compared with CTL, the CLA treatment increased NEB by 77.5% for wk 8, which was before ISOP challenge. Baseline plasma concentrations (−20 and −5 min before the ISOP challenge) of glucose, NEFA, and BHBA were not affected by CLA treatment (Table 4). The baseline concentration of insulin was numerically higher in the CLA group than in the CTL group ($P = 0.15$). Compared with the CTL, the glucose-to-insulin ratio at baseline tended to be lower in the CLA treatment ($P = 0.08$). After ISOP challenge, concentrations of glucose, NEFA, and insulin increased for 5 or 10 min and decreased thereafter to return to baseline concentrations at 25 or 30 min (Figure 2). The maximal concentrations (peak) and the increases above basal concentrations of glucose, NEFA, and insulin were not affected by CLA treatment (Table 4). The concentration of BHBA decreased during the 60 min after ISOP injection.

Changes in the AUC for glucose, NEFA, BHBA, insulin, and total AUC of glucose and NEFA were not affected by CLA treatment. Total AUC of BHBA and insulin tended to be higher ($P = 0.10$ and 0.13, respectively) and the ratio of total AUC of glucose to total

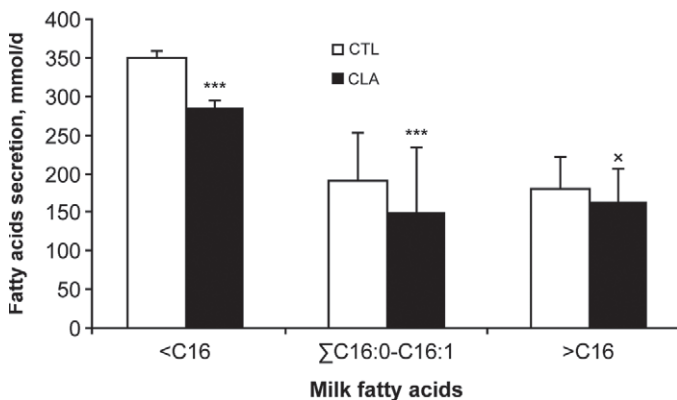


Figure 1. Milk FA secretion (mmol/d) in lactating goats fed 45 g/d of Ca salts of palm oil (control, CTL) or 45 g/d of conjugated linoleic acid (CLA; mixture of 2 isomers: *trans*-11,*cis*-9 C18:2 and *trans*-10,*cis*-12 C18:2). Fatty acids are categorized according to metabolic origin: <C16 synthesized de novo, C16:0 + C16:1, and >C16 taken up from circulating plasma lipids. Values presented are least squares means, with data measured during wk 2 as covariates. *** $P < 0.001$; × indicates $P = 0.07$. Error bars indicate standard deviations.

Table 4. Changes in plasma concentrations of glucose, NEFA, BHBA, and insulin to isoproterenol (ISOP) challenge (4 nmol of ISOP/kg of BW) in lactating goats fed 45 g/d of Ca salts of palm oil (control, CTL) or 45 g/d of conjugated linoleic acid (CLA; mixture of 2 isomers: *trans*-11,*cis*-9 C18:2 and *trans*-10,*cis*-12 C18:2)¹

Item	Treatment		SEM	P-value
	CTL (n = 6)	CLA (n = 6)		CLA
Net energy balance, kcal/d	373	662	148	0.03
BW, kg	61.63	62.82	2.93	0.85
Baseline concentration				
Glucose, g/L	0.586	0.601	0.019	0.72
NEFA, μmol/L	178.3	157.6	14.24	0.49
BHBA, mg/L	42.3	51.1	3.39	0.21
Insulin, μg/L	0.236	0.415	0.06	0.15
Glucose:insulin ratio	3.15	1.77	0.395	0.08
Maximal concentration ²				
Glucose, g/L	0.729	0.738	0.019	0.83
NEFA, μmol/L	311.3	322.3	21.56	0.81
BHBA, mg/L	28.3	36.7	2.42	0.08
Insulin, μg/L	0.662	0.818	0.072	0.30
Increase above basal value				
Glucose, g/L	0.128	0.151	0.020	0.58
NEFA, μmol/L	133.1	164.8	13.5	0.26
BHBA, mg/L	-14.1	-14.4	1.69	0.91
Insulin, μg/L	0.426	0.403	0.034	0.76
Changes in AUC ³				
Glucose, mg/L × 25 min	1.76	2.65	0.42	0.31
NEFA, μmol/L × 25 min	1,019.4	1,416.5	174.3	0.27
BHBA, mg/L × 25 min	-162.5	-121.2	31.07	0.53
Insulin, μg/L × 25 min	5.02	4.74	0.87	0.88
Total AUC ⁴				
Glucose, mg/L × 25 min	29.1	29.4	0.02	0.86
NEFA, μmol/L × 25 min	9,435	8,948	684	0.74
BHBA, mg/L × 25 min	1,723	2,160	133	0.10
Insulin, μg/L × 25 min	17	24	2.4	0.13
Glucose:insulin ratio, ⁵ × 25 min	1.88	1.38	0.16	0.11

¹Values represent mean response to ISOP (4 nmol of ISOP/kg of BW) challenge.

²The maximal value was observed at 5 or 10 min after the ISOP injection.

³Area under the curve (AUC) was calculated as the positive incremental area that was above baseline value during the first 25 min after injection.

⁴Total AUC was calculated as the area from -20 into 25 min relative to ISOP injection.

⁵Ratio of total AUC of glucose:total AUC of insulin (between -20 and +25 min relative to ISOP injection).

AUC of insulin tended to be lower ($P = 0.11$) in the CLA group compared with the CTL (Table 4).

DISCUSSION

Animal Performance

Compared with cows (reviewed by Harvatine et al., 2009; Bauman et al., 2011), dietary CLA have been less used in dairy goats (Erasmus et al., 2004; Lock et al., 2008; Shingfield et al., 2009). Our data confirm previous conclusions demonstrating that milk fat yield is reduced after CLA supplementation in goats but that goats appear less sensitive to CLA than cows (Shingfield et al., 2009). Indeed, we obtained a mean proportion of 0.10% *trans*-10,*cis*-12 C18:2 in milk fat that decreased MFC by 15% and fat yield by 18%. In comparison, a similar proportion of *trans*-10,*cis*-12 C18:2 in milk induced a

decrease in milk fat content and in milk yield between 25 and 30% in cows (Perfield et al., 2004; Moore et al., 2005). Moreover, temporal patterns of decrease in MFC are different between goats and cows, as the response to CLA is obtained within 2 to 3 d in cows, whereas we found it takes at least 7 d in goats (see also Lock et al., 2008).

In our experiment, the decrease in milk fat content and fat yield were intermediary to those previously reported in goats (Lock et al., 2008; Shingfield et al., 2009). The main difference in response is largely related to the *trans*-10,*cis*-12 C18:2 intake and its bioavailability. Using a dose ranging from 7 to 22 g of *trans*-10,*cis*-12 C18:2/d (Shingfield et al., 2009) and from 3 to 6 g/d (Lock et al., 2008), respectively, the mean concentration of *trans*-10,*cis*-12 C18:2 in milk ranged from 0.2 to 0.8%, and from 0.1 to 0.2% respectively. In those studies, the calculated transfer efficiency of di-

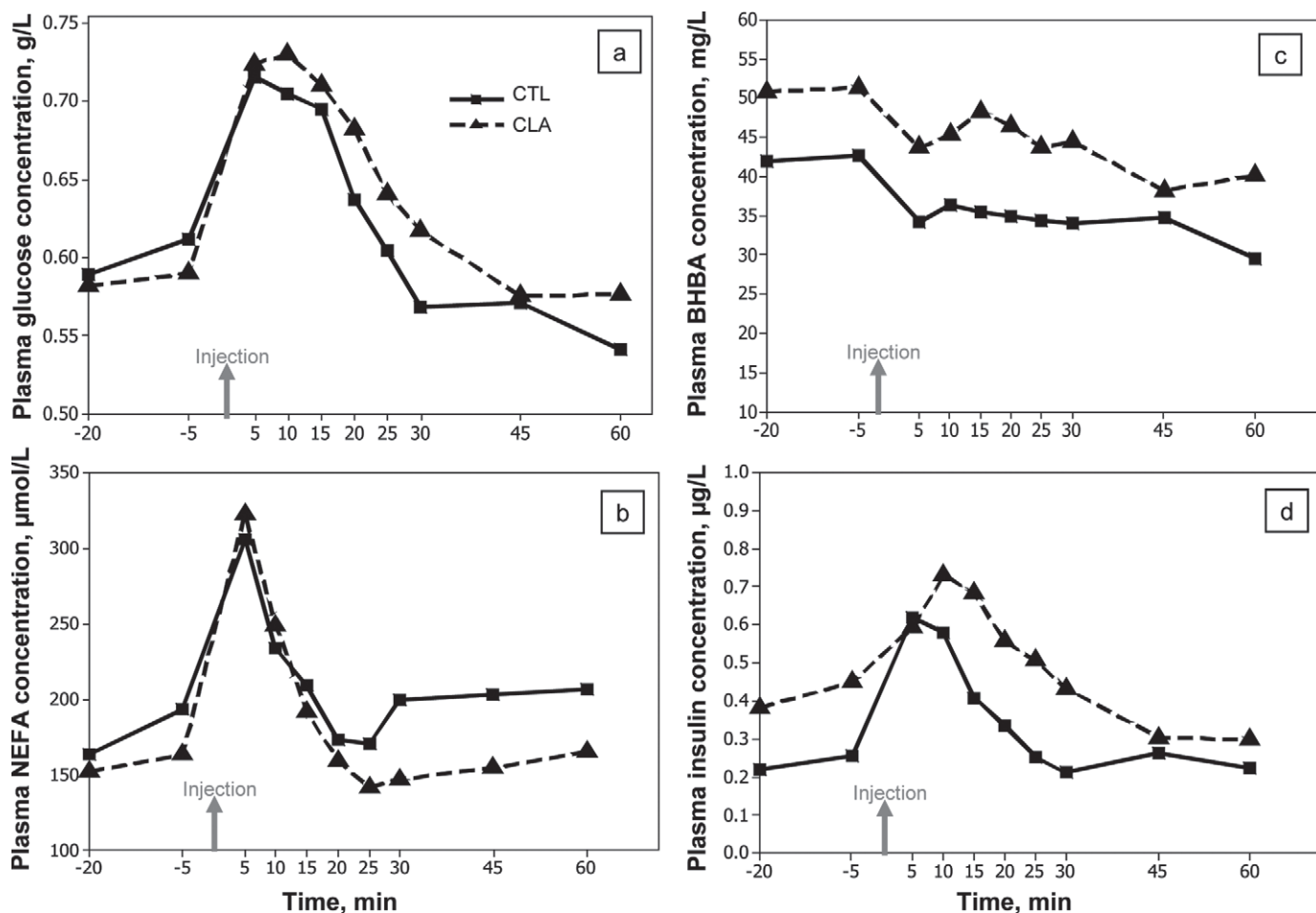


Figure 2. Mean plasma concentrations (a: glucose, b: NEFA, c: BHBA, and d: insulin) in response to isoproterenol challenge (4 nmol/kg of BW) in lactating goats fed 45 g/d of Ca salts of palm oil (control, CTL) or 45 g/d of conjugated linoleic acid (CLA; mixture of 2 isomers: *trans*-11, *cis*-9 C18:2 and *trans*-10, *cis*-12 C18:2). Standard errors of the means for glucose, NEFA, BHBA, and insulin were 0.014 g/L, 16.82 μ mol/L, 2.65 mg/L, and 0.086 μ g/L, respectively.

etary *trans*-10, *cis*-12 C18:2 to milk was between 1.7 and 2.4% (Shingfield et al., 2009) and between 1.8 and 1.9% (Lock et al., 2008). This was in line with the value in the present study (1.9%). The corresponding decreases in MFC were 25 to 36% and 5 to 18% for Shingfield et al. (2009) and Lock et al. (2008), respectively. The intake of 4.5 g of *trans*-10, *cis*-12 C18:2/d in our experiment produced a 15% decrease in MFC. However, closer comparison with the results of Lock et al. (2008) shows that at a similar *trans*-10, *cis*-12 proportion in milk, we obtained a greater decrease in MFC and in fat yield. This suggests that CLA-induced decrease in MFC is affected by the initial level of milk fat (31.2 g/L in Lock et al., 2008 vs. 45 g/L in our trial). In line with this, at similar levels of *trans*-10, *cis*-12 C18:2 intake, the decrease in milk fat was greater in goats fed supplements of FA than those fed no lipid supplement (Shingfield et al., 2009). Similarly, the response of MFC to

CLA was less pronounced in cows exhibiting low initial MFC than those with high MFC (i.e., when cows were fed high-concentrate compared with low-concentrate diets; Glasser et al., 2010). In our conditions, a high-concentrate diet based on corn silage led to high MFC and it elicited a significant decrease in MFC in goats fed low doses of *trans*-10, *cis*-12 C18:2.

Our results also show that supplementation with 4.5 g of *trans*-10, *cis*-12 C18:2/d in lactating goats fed a high-concentrate diet based on corn silage had no effect on DMI, BW, milk yield, or milk protein and lactose yields and contents throughout a 7-wk trial. This agrees with data in goats supplemented with a wide range of doses of *trans*-10, *cis*-12 C18:2 and fed alfalfa or corn silage diets with 35 to 60% concentrate (Lock et al., 2008; Shingfield et al., 2009).

Because CLA treatment induced a decrease in the net energy needs due to a reduction in milk fat secre-

tion (at constant DMI, BW, and milk yield relative to the CTL), CLA supplementation gradually increased net energy balance. We observed a mean increase in NEB of 260 Kcal/d between CLA and CTL treatments, larger than the calculated increase in NEB of 60 of Lock et al. (2008) and 90 Kcal/d of Shingfield et al. (2009). These differences may be related to the fact that the above-mentioned studies in goats were conducted during late lactation (140 to 250 d postpartum), which can affect the CLA-induced changes in partitioning of energy nutrients away from mammary gland (Gervais et al., 2005).

Response to an Isoproterenol Challenge

Before ISOP injection, plasma NEFA concentrations were not affected by CLA treatment, as reported in cows in positive energy balance or during established lactation (Baumgard et al., 2002; de Veth et al., 2006). Plasma NEFA concentration is an index of basal fat mobilization that reflects the apparent balance between basal lipolysis of triglyceride and FA esterification rate or de novo lipogenesis in adipose tissue. These processes are known to be regulated by insulin that decreases lipolysis and increases reesterification and de novo lipogenesis (Brockman and Laarveld, 1986). Insulin may control NEFA appearance through enhanced trapping into the adipose tissue of NEFA derived from intravascular triglyceride lipolysis (Carpentier et al., 2005). Conversely to data in dairy cows where no change in plasma insulin concentration were reported after CLA treatment (de Veth et al., 2006; Kay et al., 2006), we observed a trend for plasma insulin and insulin:glucose ratio to be higher in goats fed CLA, probably reflecting their higher NEB balance compared with CTL goats at this time. In dairy cows, the expression of lipogenic enzymes and of genes related to lipid synthesis in subcutaneous adipose tissue was increased during short-term *trans*-10, *cis*-12 C18:2 supplementation (Harvatine et al., 2009), reflecting the partitioning of spared energy away from milk toward adipose tissue. To date, no data are available reporting the effect of CLA on lipogenesis in adipose tissue of goats.

As no data are available on the measurement of lipolysis in adipose tissue of goats fed *trans*-10, *cis*-12 C18:2, we attempted to determine if β -agonist-stimulated lipolysis may have been enhanced by CLA supplementation. Isoproterenol is a synthetic catecholamine that is a lipolytic agent that stimulates both β 1- and β 2-adrenergic receptors on adipocytes (Lafontan, 2008). Thus, the NEFA response to ISOP provides an efficient method of studying the adipose tissue lipolytic potential because it induces an increase in plasma NEFA

concentration, which reflects the lipolytic properties of this β -agonist minus the fraction of released NEFA that is directly reesterified (Chilliard et al., 1998). The response to a β -agonist injection (norepinephrine) is unchanged in dairy cows fed CLA and a low-ME diet during midlactation (de Veth et al., 2006) or eventually reduced in dairy cows in positive net energy balance after CLA supplementation (Baumgard et al., 2002). In our study, no change in any of the parameters of NEFA response was observed after CLA treatment, as observed in cows (de Veth et al., 2006).

Before ISOP injection, plasma glucose concentration was not affected by CLA treatment, as observed in cows (Baumgard et al., 2002; de Veth et al., 2006). In the present study, glucose concentration increased rapidly after ISOP injection, probably because of its effects on liver glycogenolysis (Drackley et al., 2001) and possibly on liver gluconeogenesis (Faulkner and Pollock, 1990). This increase in glucose was also associated with an increase in insulin, with a maximal response at 10 min relative to ISOP injection, which is probably due to pancreas stimulation of insulin synthesis by glucose (Brockman and Laarveld, 1986; Oda et al., 1986). Nevertheless, this increase in glucose and insulin concentration after the ISOP challenge was not affected by CLA treatment in our study. The changes in AUC above baseline value and total AUC of glucose remained unaffected by CLA treatment. Nevertheless, total AUC of insulin tended to be higher and the total AUC of glucose-to-total AUC of insulin ratio tended to be lower in the CLA treatment, which may suggest increased pancreas sensitivity to hyperglycemia induced by ISOP. It is plausible that energy spared through the effects of CLA on milk energy output could affect the pancreas sensitivity to glucose to favor an increase in body fat lipogenesis (Harvatine et al., 2009).

Milk FA Secretion and Profile

The reduction in fat yield in our experiment was due to the simultaneous decrease in the mammary secretion of <C16 FA (−18%), C16 FA (−20%), and to a lesser extent, a decrease in >C16 FA secretion (−11%). This reflects a CLA-coordinated downregulation of mRNA expression of lipogenic enzymes and enzymes of lipid metabolism in the mammary gland, implicating a role for sterol response element-binding protein 1 and Spot 14 at the cellular signaling level (Bauman et al., 2011). Generally, the decrease in FA secretion is greater for short-chain and medium-chain FA than for long-chain FA, leading to a higher proportion of long-chain FA in the milk fat of cows (Maxin et al., 2011) and of sheep (Sinclair et al., 2010). Our results are in agree-

ment with these data, as CLA decreased the secretion of all FA, whatever their chain-length, but long-chain FA secretion was less reduced, which resulted in higher proportion of >C16 FA in milk fat.

However, some differences existed between our results and those previously reported in goats. With a similar concentration of *trans*-10,*cis*-12 C18:2 in goat milk, Lock et al. (2008) reported a smaller reduction in the secretion of short-chain FA and total C16 FA, and similar reductions as in our experiment were reported only with higher *trans*-10,*cis*-12 C18:2 proportion (0.3% of total FA; Shingfield et al., 2009). Moreover, >C16 FA secretion was not reduced, whatever the dose of *trans*-10,*cis*-12 C18:2 in diet in the study of Shingfield et al. (2009), and it was significantly reduced only at a high *trans*-10,*cis*-12 C18:2 concentration in milk in the study of Lock et al. (2008). Differences in lactation stage and in duration of supplementation could explain these between-experiment differences (Bauman et al., 2008). The difference in >C16 FA secretion may reflect changes in lipoprotein lipase (LPL) activity or synthesis that are differentially affected by stage of lactation, energy balance, and dietary factors (Bernard et al., 2008), as mammary LPL activity is high during midlactation and it decreased during late lactation in goats (Chilliard et al., 2003). This suggests that the ability of CLA to decrease the expression of genes involved in mammary uptake (LPL) is more effective during established lactation than during late lactation in dairy goats.

Among long-chain FA, our results are in agreement with those of Shingfield et al. (2009) and Lock et al. (2008) who reported that the reduction in milk fat synthesis in goats was associated with a decrease in the proportions of total *cis* C18:1 and an increase in those of C18:0 and total *trans* C18:1. However, in our study, the increase in total *trans* C18:1 was not significant, reflecting only small changes in individual isomers of *trans* C18:1, except for an increase in *trans*-11 C18:1 proportion. This differs from most reports in dairy cows and goats where *trans* C18:1 were generally increased. Among *cis* C18:1 FA, only the proportion of the *cis*-9 isomer was reduced, which indicates a decrease in the activity of the mammary stearoyl-CoA desaturase as reflected first by the increase in C18:0 and second by the decrease in the calculated desaturase indices ratios that are a proxy of the stearoyl-CoA desaturase activity. Reductions in mammary desaturase indices have been reported with high doses of CLA in lactating cows (Peterson et al., 2004) and sheep (Sinclair et al., 2010), but not at low dosage (Maxin et al., 2011), although this is not a prerequisite for the decrease in milk fat yield (Peterson et al., 2004; de Andrade and Schmidely, 2006).

CONCLUSIONS

Conjugated linoleic acid supplementation (45 g/d) in goats fed a high-concentrate diet based on corn silage had no effect on DMI, BW, milk yield, or milk protein and lactose yields and contents, but it increased NEB. Conjugated linoleic acid supplementation significantly decreased milk fat content and yield, the secretion of short-chain and medium-chain FA, and to a lesser extent, the secretion of long-chain FA. In goats that had adequate energy intake in midlactation, the response of adipose tissue to an ISOP challenge was unaltered by CLA.

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APPENDIX

Table A1. Milk FA composition (g/100 g of milk fat) in lactating goats fed 45 g/d of Ca salts of palm oil (control, CTL) or 45 g/d of conjugated linoleic acid (CLA; mixture of 2 isomers: *trans*-11,*cis*-9 C18:2 and *trans*-10,*cis*-12 C8:2)¹

Item	Treatment			P-value		
	CTL (n = 12)	CLA (n = 12)	SEM	CLA	Time (T)	CLA × T
C4:0	2.24	2.52	0.08	0.03	0.39	0.12
C6:0	2.92	2.80	0.09	0.16	0.20	0.18
C8:0	3.55	3.27	0.11	0.001	0.71	0.27
C10:0	12.27	11.26	0.36	0.005	0.27	0.16
C11:0	0.31	0.22	0.02	0.001	0.33	0.29
C12:0	5.21	4.68	0.23	0.07	0.06	0.13
C13:0	0.16	0.12	0.01	0.04	0.70	0.73
C14:0	9.56	10.56	0.18	0.006	0.13	0.98
C15:0	0.83	0.88	0.03	0.21	0.76	0.39
C16:0	27.99	26.59	0.79	0.05	0.005	0.55
C17:0	0.60	0.62	0.02	0.31	0.27	0.66
C18:0	6.55	9.45	0.35	<0.0001	0.11	0.37
C19:0	0.13	0.15	0.03	0.02	0.07	0.92
C20:0	0.11	0.13	0.005	0.0003	0.35	0.04
C21:0	0.02	0.02	0.03	0.81	0.0001	0.63
C22:0	0.03	0.05	0.004	0.001	0.001	0.84
<i>cis</i> -5 C14:1	0.11	0.09	0.007	0.003	0.51	0.87
<i>cis</i> -6 C15:1	0.17	0.18	0.03	0.36	<0.0001	0.47
<i>cis</i> -7 C16:1 + <i>anteiso</i> C17:0	0.82	0.77	0.02	0.08	0.15	0.59
<i>cis</i> -9 C18:1	12.54	10.93	0.02	0.001	0.43	0.68
<i>cis</i> -11 C18:1	0.45	0.44	0.02	0.39	0.39	0.09
<i>cis</i> -12 C18:1	0.28	0.28	0.005	0.78	0.84	0.82
<i>cis</i> -13 C18:1	0.10	0.10	0.01	0.54	<0.0001	0.44
<i>cis</i> -15 C18:1	0.06	0.06	0.005	0.54	<0.0001	0.35
<i>trans</i> -4 C18:1	0.04	0.04	0.002	0.30	0.79	0.54
<i>trans</i> -5 C18:1	0.04	0.04	0.02	0.32	0.11	0.80
<i>trans</i> -6,-7,-8 C18:1	0.39	0.40	0.01	0.72	0.54	0.39
<i>trans</i> -9 C18:1	0.31	0.32	0.12	0.88	<0.0001	0.17
<i>trans</i> -10 C18:1	0.93	0.60	0.29	0.17	0.94	0.52
<i>trans</i> -11 C18:1	2.56	3.52	0.009	0.06	0.68	0.17
<i>trans</i> -12 C18:1	0.35	0.38	0.04	0.11	0.96	0.58
<i>trans</i> -13,-14 C18:1	0.43	0.50	0.45	0.06	0.0005	0.91
<i>trans</i> -15 C18:1	0.32	0.33	0.01	0.45	0.46	0.30
<i>trans</i> -16 C18:1	0.18	0.22	0.004	0.08	0.75	0.23
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.82	2.71	0.004	0.42	0.60	0.14
<i>cis</i> -9, <i>trans</i> -12 C18:2	0.08	0.07	0.01	0.13	<0.0001	0.06
<i>trans</i> -9, <i>cis</i> -12 C18:2	0.05	0.05	0.005	0.60	0.65	0.84
<i>trans</i> -11, <i>cis</i> -15 C18:2	0.08	0.07	0.08	0.58	0.65	0.293
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.91	0.96	0.1	0.76	0.51	0.18
<i>trans</i> -10, <i>cis</i> -12 C18:2	ND ²	0.098	0.001	0.0001	0.94	0.98
C18:3n-6	0.02	0.02	0.02	0.76	0.002	0.21
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.27	0.28	0.0003	0.76	0.0002	0.40
<i>cis</i> -11 C20:1	0.06	0.06	0.06	0.46	0.0003	0.93
C20:3n-3	0.10	0.08	0.082	0.007	0.003	0.98

¹Values presented are least squares means, with data measured during wk 2 as covariates.²ND = not detected.