The effect of dietary rumen-protected *trans*-10,*cis*-12 conjugated linoleic acid or a milk fat-depressing diet on energy metabolism, inflammation, and oxidative stress of dairy cows in early lactation

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ABSTRACT

The objective of this study was to determine the effects of milk fat depression induced by supplementing conjugated linoleic acid (CLA; *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA) or feeding a higher starch and oil-containing diet (HSO) on metabolic changes in dairy cows after calving. The main hypothesis was that the 2 strategies to decrease milk fat yield could have different effects on performance, energy balance (EB), and inflammatory status in early lactation. Thirty-three Nordic Red dairy cows were used in a randomized block design from 1 to 112 d of lactation and fed one of the following treatments: control (CON), CLA-supplemented diet, or HSO diet. Dry matter intake and milk yield were measured daily whereas milk composition was measured weekly throughout the experiment. Nutrient digestibility, EB, and plasma hormones and metabolites were measured at 3, 7, 11, and 15 wk of lactation in respiration chambers. The HSO diet led to lower intakes of dry matter, neutral detergent fiber, and gross energy compared with CON and CLA diets. The CLA diet and especially the HSO diet resulted in lower energy-corrected milk yield during the first 7 wk of lactation than those fed CON. The EB was numerically higher for HSO and CLA diets compared with CON at wk 3 and 7. Plasma glucose concentration was higher by the CLA diet at wk 3 and by the HSO diet from wk 3 to 15 compared with CON. Plasma nonesterified fatty acids were higher at wk 3 in the CON group (indicating more lipid mobilization) but decreased thereafter to similar levels with the other groups. The HSO-fed cows had higher plasma ceruloplasmin, paraoxonase, and total bilirubin concentrations in the entire experiment and showed the highest levels of reactive oxygen metabolites. These results suggest an increased inflammatory and oxidative stress state in the HSO cows and probably different regulation of the innate immune system. This study provides evidence that milk fat depression induced by feeding HSO (as well as CLA) decreased milk fat secretion and improved EB compared with CON in early lactation. The increase in plasma glucose and paraoxonase levels with the HSO diet may imply a better ability of the liver to cope with the metabolic demand after parturition. However, the negative effect of HSO on feed intake, and the indication of increased inflammatory and oxidative stress warrant further studies before the HSO feeding strategy could be supported as an alternative to improve EB in early lactation.

Key words: conjugated linoleic acid, energy metabolism, milk fat depression, postpartum

INTRODUCTION

Feed intake is often inadequate during early- to mid-lactation to support the rapid increase in energy required for milk production resulting in negative energy balance (**EB**) affecting health, production, and reproductive performance (Grummer, 1993). Increasing energy intake by feeding lipogenic nutrients (such as palmitic or oleic acids) increases milk fat production, which may not be always beneficial as indicated by more negative EB (de Souza et al., 2021) and higher BW loss (Beam and Butler, 1998) in the early lactation period. On the other hand, isocaloric diets with a greater ratio of glucogenic to lipogenic nutrients decreased milk fat concentration and yield and increased energy partitioning toward body gain which have been associated with improved EB of dairy cows in early and

post-peak lactation (van Knegsel et al., 2007; Boerman et al., 2015).

Milk fat depression (**MFD**) might improve negative EB during the transition period by partitioning the energy spared from milk fat synthesis toward body reserves (Moore et al., 2004; Harvatine et al., 2009), especially if feed intake (Boerman et al., 2015), health conditions, and immune or oxidative status (Mazzetti et al., 2020) are not compromised. A maximum 50% reduction in milk fat synthesis has been observed during diet-induced MFD (i.e., feeding high-concentrate or high PUFA diets; Bauman and Griinari, 2003) and in dose-response studies with abomasal infusion of *trans*-10,*cis*-12 CLA isomer (Baumgard et al., 2002; de Veth et al., 2004; Haubold et al., 2020). *Trans*-10,*cis*-12 CLA is a biohydrogenation (**BH**) intermediate known to inhibit milk fat synthesis (Bauman and Griinari 2003), but diet-induced MFD can be mediated through production of specific *trans*-BH intermediates (Ventto et al., 2017). The relationship between MFD and repartitioning of energy induced by abomasal infusion of *trans*-10,*cis*-12 CLA has been highlighted in recent studies (Haubold et al., 2020; Vogel et al., 2020). However, the effects of dietary CLA supplements on EB and feed intake during early lactation are inconsistent (Bernal-Santos et al., 2003; Moallem et al., 2010; Schäfers et al., 2017).

Almost all periparturient dairy cows experience some degree of immune activation and inflammation, which affect or impair the progress of many metabolic pathways (Bionaz et al., 2007; Bertoni and Trevisi, 2013; Trevisi and Minuti, 2018), thereby increasing the occurrence of metabolic and infective diseases in the postcalving period (Trevisi et al., 2014b; Horst et al., 2021). Several acute phase proteins (**APP**) are released from liver hepatocytes as part of a general nonspecific inflammatory response (Bertoni and Trevisi, 2013). Hence, this condition can affect the liver function and cause negative effects on synthesis of albumin, apolipoproteins, hormone carriers, and enzymes of many metabolic pathways (Trevisi et al., 2012). The effects of rumen-protected CLA on systemic inflammatory status (Trevisi et al., 2008; Haubold et al., 2020) have not been conclusive. An inverse association between inflammation status during the first month of lactation and liver function has been reported. Circulating proinflammatory cytokines could represent a common mechanism linking disease or health disorders with impaired liver function especially in association with negative EB (Bertoni et al., 2008). It has been shown that the increases in oxidative stress and inflammation during early lactation as assessed by reactive oxygen metabolites (**ROM**) and APP, respectively, are related to greater glucose demand and reduced liver functionality (Bertoni and Trevisi, 2013).

The present study was planned to better understand the effects of a diet-induced MFD (producing a wide range of BH intermediates rather than only *trans*-10,*cis*-12 CLA) in early lactation on energy metabolism and inflammation responses, compared with CLA-induced MFD. We formulated a diet containing free oil rich in PUFA that provides BH intermediates and different CLA isomers, which might act as immune system mediators and affect the inflammation responses differently from *trans*-10,*cis*-12 CLA as described by Minuti et al. (2015).

In our previous publication from this experiment (Qin et al., 2018), we evaluated the adipose tissue gene expression, and were able to demonstrate different mechanisms for regulating lipid metabolism in adipose tissue with respect to insulin signaling pathways. The main objective of the current study was to determine how these 2 different approaches inducing MFD would differ with regards to EB and inflammo-metabolic conditions with emphasis on production responses, nutrient digestibility, and energy metabolism in the early postpartum period. In addition, blood metabolites and hormones involved in energy and lipid metabolism were measured to allow us to better identify the possible mode of actions of the treatments. We hypothesized that a higher starch diet containing PUFA inducing MFD would improve EB of dairy cows during early lactation, have positive effects on health and inflammo-metabolic status and thereby provide a feasible alternative to rumen-protected CLA supplementation (*trans*-10,*cis*-12 CLA) for improving the energy status of dairy cows in early lactation.

MATERIALS AND METHODS

Cows, Experimental Design, and Treatments

Animal care and all experimental procedures used in this study were approved by the National Ethics Committee (ESAVI/4997/04.10.03/2012, Hämeenlinna, Finland) in accordance with the guidelines established by the European Community Council Directives 86/609/EEC. The study was conducted as a randomized block design recruiting 33 multiparous Nordic Red dairy cows. All cows were in good health at the beginning of the trial, and health protocols were maintained during this study. The cows with (mean ± SD) 792 ± 72 kg of BW during 14 d before calving and 3.0 ± 1.1 parity were recruited from 28 d before anticipated calving date until 112 d postpartum. Animals received the experimental diets immediately after calving and were blocked by expected calving date, BW, parity, and Nordic total merit (https://www.nordicebv.info/ntm-nordic -total-merit). Blocks were completed sequentially dur-

ing 2012 to 2013, and each block consisted of 6 cows calving rather simultaneously (2 cows per treatment). Within each block, cows were randomly assigned to 3 experimental treatments. In our previous paper (Qin et al., 2018), we presented data from 30 cows of the same experiment (792 ± 72 kg of BW and 2.9 ± 1.0 parity) on gene expression involved in lipid metabolism in adipose tissue and some blood metabolites at 3 and 15 wk of lactation. In addition, some of the production variables and EB as calculated by subtracting estimated ME requirement ($BW^{0.75} \times 0.515 + 5.15 \times ECM$) from ME input were reported. The cows were housed in an experimental freestall barn (Jokioinen, Finland), had free access to water and salt block, and were milked twice daily at 0700 and 1645 h either in milking parlor or in situ (during EB measurements).

During the prepartum period, 21 d before expected calving date, the cows were fed a standard prepartum concentrate. The cows had free access to grass silage and received 1 to 4 kg/d concentrate (% DM: 44.4 rolled barley, 26.7 solvent-extracted rapeseed meal, 11.1 molassed-sugar beet pulp, 11.1 barley feed, and 6.7 mineral–vitamin premix). The concentrate allocation was 1 kg/d on d 28 before expected calving and gradually increased to 4 kg/d on d 7 before calving and remained constant until calving. Prepartum concentrate chemical composition (% DM) was 18.8 CP, 25.1 NDF, and 32.7 starch. Treatments consisted of (1) a basal diet based on grass silage (**CON**; n = 13), (2) the same basal diet supplemented with a CLA supplement (CLA diet; n = 9), which supplied 10 to 15 g/d of *trans*-10,*cis*-12 and an equal amount of *cis*-9,*trans*-11 CLA, and (3) a grass silage-based diet with a higher starch content (20.2% DM), 33.3% DM of dietary NDF, and supplemented with 4.0% DM of a mixture of UFA (**HSO**; n = 11, Table 1) to induce 15% depression in milk fat yield based on our previous experiments (Kairenius, 2020; Darabighane et al., 2021). In our previous experiment (Darabighane et al., 2021), a high starch diet supplemented with 3% sunflower oil and fish oil (2:1) caused 12% depression in milk fat and to ensure stronger MFD (at least 15%), the oil content was increased to 4% of diet DM in the current experiment. The CLA and HSO diets were formulated to induce a decrease in milk fat yield during the first 112 d of lactation. The MFD-inducing HSO diet was obtained by lowering nonforage NDF, increasing starch concentration from 16.4 to 20.2% of diet DM by replacing rolled barley grain, sugar beet pulp, and barley feed with wheat grain and a mixture of UFA compared with CON and CLA groups. The rumen-unprotected UFA mixture consisted of sunflower oil [61.2 g linoleic acid /100 g fatty acids (**FA**); EBM Grupp AS] and fish oil (27.0 g

of very long-chain PUFA /100 g of FA; BASF) in a 2:1 ratio (wt/wt), which replaced concentrate ingredients as described above (Table 1). The rumen-protected, lipid-encapsulated CLA supplement contained both *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers of CLA at equal contents of 10.0 ± 2.0% (Lutrell Pure, BASF). To achieve a certain level of ruminal protection, the liquid form of CLA, Lutalin, was mixed with hot high-melting fat (hydrogenated soybean oil) before spray drying. The CLA supplement contained 12.2% palmitic acid, 52.2% stearic acid, 9.0% oleic acid, and 0.8% linoleic acid. The CLA supplement, as a granule form, was top dressed in 2 equal portions daily at 0630 and 1830 h at 150, 125 and 100 g/d during d 1 to 14, 15 to 21, and 22 until the end of study, which resulted in providing on average 15.0, 12.5, and 10.0 g of each isomer per day, respectively. The adjustment of CLA supplementation over time was done to compensate for the lower sensitivity of mammary gland to the inhibitory effects of *trans*-10,*cis*-12 CLA at the onset of lactation (Moore et al., 2004). We anticipated that a maximum reduction in milk fat yield occurs at a dose of 10 g/d *trans*-10,*cis*-12 CLA as explained by de Veth et al. (2004). All the cows received 600 g of CON concentrate per day when visiting milking parlor (excluding EB measurement times).

The forage was restrictively fermented grass silage prepared from primary growth of mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards, grown at Jokioinen (60°49′N, 23°28′E) and treated with a formic acid-based ensiling additive (5 L/tone, AIV 2 Plus, Valio Ltd.). The diets were offered as a TMR to avoid selection of dietary components as 4 meals at 0630, 1300, 1600, and 1830 h, had a forage-to-concentrate ratio of 55:45 on DM basis, and were formulated to be as iso-protein as possible. The proportion of grass silage was similar for all experimental diets, whereas the amounts of other ingredients were different among the CON or CLA diets and HSO diet. The experimental diets were offered ad libitum to result in 5 to 10% refusals and formulated to meet requirements for metabolizable energy and protein of lactating cows producing 40 kg of milk/d (MTT Agrifood Research Finland, 2006). The ingredients and chemical composition of postpartum diets are presented in Table 1.

Experimental Measurements and Sampling

Methane, carbon dioxide, and hydrogen production and oxygen consumption were measured by 4 open-circuit respiratory chambers (288 × 396 × 220 cm) where animals were housed individually as described by Bayat et al. (2022b). Experimental feeds were of-

fered ad libitum 4 times daily at 0600, 0900, 1600, and 1900 as TMR. Energy balance was measured over a 5-d period starting on average 14 d postpartum when the first day was considered as acclimatization. The measurements were repeated at 28-d intervals (wk 3, 7, 11, and 15). Feed intake and the output of feces, urine and milk for half of the cows from each block were measured in the chambers (16 cows in total) and for the other half in dedicated tie stalls for total collection of feces and urine (17 cows in total) during 4 d of each balance period after 1 d of acclimatization.

Daily feed intake determined as offered TMR minus refusals and milk yield were measured throughout the study but only the data during EB measurements were involved in statistical analysis. Silage and concentrate samples were collected on a weekly basis and stored frozen (−20°C) until chemical analysis, following standard

Table 1. Formulation and chemical composition of experimental diets containing the CLA supplement or higher starch and oil[1]

| Item | Diet[2] | |
	CON	HSO
Feed ingredient, % of diet DM		
Grass silage[3]	55	55
Rolled barley	21	6.9
Ground wheat	—	20.9
Molassed-sugar beet pulp	5.5	—
Barley feed	5.5	—
Rapeseed meal, solvent-extracted	11.5	11.5
Urea	—	0.2
Mixture of UFA[4]	—	4
Vitamin and mineral premix[5]	1.5	1.5
Chemical composition, % of DM		
unless otherwise stated		
DM, % as fed	54.8	55.1
OM	92.5	93.0
CP	15.9	15.7
NDF	37.9	33.3
Total fatty acids	2.47	5.96
Starch	16.4	20.2
Water-soluble carbohydrates	4.38	3.71
Gross energy, Mcal/kg of DM	4.30	4.49

[1]The table is reprinted from Qin et al. (2018).

[2]CON = control diet and HSO = a higher starch (20.2% DM) diet containing 4.0% mixture of UFA.

[3]Mean fermentation characteristics of experimental silage: pH, 3.98; in DM (%) lactic acid, 5.64; acetic acid, 2.06; propionic acid, 0.049; butyric acid, 0.056; soluble N (% total N), 51.4; ammonium N (% total N), 3.91; gross energy (Mcal/kg of DM), 4.23.

[4]Containing sunflower and fish oils (2:1, wt/wt). Sunflower oil contained (g/100 g of fatty acids) 16:0 (6.1), 18:0 (3.6), 18:1n-9 (25.4), and 18:2n-6 (61.2). Fish oil contained (g/100 g fatty acids) 14:0 (7.3), 16:0 (16.0), 16:1n-7 (7.8), 18:1n-9 (8.3), 20:5n-3 (16.5), and 22:6n-3 (10.5).

[5]Declared as containing (%) calcium (19.0), magnesium (6.0), sodium (13.5), zinc (0.219), manganese (0.045), copper (0.040); (mg/kg) iodine (55), cobalt (35), selenium (30), and dl-tocopheryl acetate (550); (IU/kg) retinyl acetate (220,000), and cholecalciferol (40,000), Onni, Melica Finland Ltd.

methods described by Shingfield et al. (2001). Silage DM content and consequently DMI were corrected for the loss of volatiles according to Huida et al. (1986). Body weight was measured before morning and afternoon milking throughout the experiment excluding EB measurement days. To minimize the daily variation, the average values of 3 consecutive days before and after respiration chamber measurement times were used to calculate the average BW. Daily BW change was used based on the average BW calculated for each EB measurement. Body condition score (based on a 5-point scale, where 1 = thin and 5 = fat; Wildman et al., 1982) was determined during the day before and after each EB measurement and the average value was used. Daily representative feces and urine subsamples (5%) for each cow were collected over 4 d, pooled within each measurement at 3, 7, 11, and 15 wk and stored at −20°C. At the end of each EB measurement subsamples of feces and urine were composited and submitted for DM, gross energy (**GE**), and nitrogen measurements. Fecal chemical analysis in dried samples was performed using the same procedures used for feeds. Concentration of GE in samples of silage, concentrates, lipid supplements, urine, and feces was determined by bomb calorimetry (1108 Oxygen bomb, Parr Instrument). Milk samples were collected over 3 d/wk (Monday, Wednesday, and Friday) for each cow, stored in tubes containing Bronopol (Valio Ltd.), pooled weekly for each cow according to morning and evening milk yields, and refrigerated at 4°C for determination of fat, CP, and lactose using a MilkoScan (MilkoScan 133B, Foss).

Blood samples were collected from a jugular vein into vacuum tubes containing sodium heparin or EDTA, or into serum tubes (Vacutainer, Becton Dickinson) at 3 d after each EB measurement according to analytical requirements described hereafter. Plasma and serum were recovered by centrifugation at 2,083 × g for 15 min at 4°C and stored at −80°C. Commercial routine analyses of blood concentrations of IGF-I, somatotropin, glucagon, and leptin based on bovine-specific ELISA assays validated by bovine samples were performed on EDTA plasma samples (Laboklin GmbH & Co.). Analysis of blood insulin concentration was performed on serum samples using the RIA kit (PI-12K, Millipore Oy), following the protocol described by Salin et al. (2012). Blood metabolites [glucose, cholesterol, triglyceride, BHB, nonesterified fatty acids (**NEFA**), creatinine, urea, and albumins], inflammatory markers (ceruloplasmin, haptoglobin, total bilirubin, and paraoxonase), and oxidative status [ROM, ferric reducing antioxidant power (**FRAP**) as a measure of the total antioxidant power, aspartate aminotransferase or glutamic-oxalo-acetic transaminase, and gamma glutamyl transferase]

were analyzed from plasma samples obtained from the jugular vein into 10-mL heparinized vacuum tubes, using the methods described by Piccioli-Cappelli et al. (2014) and Calamari et al. (2016).

Calculations and Statistical Analysis

Energy intake and energy excretion in feces and urine were calculated by multiplying DMI or excreted feces and urine DM contents by their respective GE contents. Yield of ECM was calculated based on the yields of fat, CP, and lactose {ECM = milk (kg/d) × [38.3 × fat (g/kg) + 24.2 × CP (g/kg) + 16.54 × lactose (g/kg) + 20.7] / 3,140} and energy secretion in milk was calculated by multiplying ECM by 3.14 (Sjaunja et al., 1990). Heat production of the cows was calculated based on the exchanged oxygen, carbon dioxide, and methane gases, and urinary nitrogen excretion measured in the respiratory chambers (Brouwer, 1965). Methane energy was calculated using the conversion factor 55.24 kJ/g (Kriss, 1930). Energy balance was calculated as the difference between energy intake and energy excretion as feces, urine, methane, milk, and heat measured during EB measurements. Energy metabolism was measured using indirect open-circuit respiratory chambers for half of the cows (n = 16) whereas for the rest of the cows (n = 17), it was estimated based on the same partitioning of GE intake for methane and heat production as their counterparts within each diet and chamber period in addition to the measured feed intake, milk yield, and total feces and urine DM contents in the metabolic units. Nitrogen balance was calculated as the difference between intake and feces, urine, and milk excretions.

Data were analyzed using proc GLIMMIX of SAS (version 9.4; SAS Institute Inc.) with fixed effects of block, treatment, and the interaction of time and treatment, with time as a random effect with RSIDE option in the model. Average BW during 2 wk before calving of cows was used as a covariate in the model as high correlations between BW before and after calving, and consequently with feed intake or milk yield after calving is expected (Handcock et al., 2019). For blood metabolites, hormones, and inflammation markers, the respective measurements at 21 d before expected calving were used as covariates in the model. The degree of freedom was estimated with the Kenward-Roger method. Three variance-covariance structures (autoregressive type 1, compound symmetry, and Toeplitz) were tested, and the covariance structure that minimized the Schwarz's Bayesian information criterion (i.e., compound symmetry) was chosen. When effects of treatment, time or the interaction of treatment by time were significant, the least squares means were compared using Fisher's protected LSD test with the PDIFF option. Statistical

significance was defined as $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered as a trend.

RESULTS

Nutrient Intakes and Digestibility, and Milk Yield and Composition

During chamber measurements, the HSO diet lowered intakes of ($P \leq 0.01$) DM, OM, CP, NDF, and GE from wk 3 to 15 compared with the CON and CLA groups (Table 2), whereas starch intake was similar for all 3 groups except for wk 11. Overall, DM, CP, NDF, starch, and GE intakes increased ($P \leq 0.01$) from wk 3 to 15 of lactation in all diets, whereas the increase was less noticeable for cows fed the HSO diet ($P = 0.068$ for interaction between treatment and time). Weekly average of DMI was affected by the interaction of treatment and week of lactation ($P < 0.01$; Supplemental Figure S1, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a). The HSO group had greater ($P \leq 0.01$) digestibility of OM and GE compared with other treatments throughout the experiment (Supplemental Table S1, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a). In addition, in the CLA group, mean CP digestibility was lower ($P < 0.05$) than that for HSO (69.1 vs. 70.8%) but was similar to the CON group. Treatments did not affect NDF digestibility in our study. Moreover, interactions among treatment and time were not significant for nutrient digestibility.

During chamber measurements, the milk yield was not affected by diet during the first 11 wk of lactation whereas HSO tended to decrease ($P < 0.10$) milk yield at wk 15 compared with the CLA group (38.3 vs. 42.3 kg/d; Figure 1). However, the interaction between diet and time was detected in milk yield ($P < 0.01$) in which the CLA group showed a higher milk yield at wk 15 than the HSO group. Weekly average of milk yield was not affected by treatments whereas the effects of week of lactation and its interaction with treatment was significant ($P < 0.01$; Supplemental Figure S2, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a). During chamber measurements, milk fat concentration and yield, and ECM yield were the highest for CON, intermediate for the CLA group, and the lowest for the HSO group ($P < 0.01$; Figure 1). Milk fat concentration was more stable over weeks of lactation for HSO compared with other treatments ($P < 0.01$ for interaction between treatment and time). The HSO diet decreased ($P \leq 0.01$) milk protein yield and content and increased ($P < 0.05$) lactose content compared with the CON group during wk 3 and 7. An interaction between treatment and time effects ($P < 0.01$) was observed on milk protein content as it decreased for the CON and CLA

groups from wk 3 to 7 more remarkably compared with the HSO group.

Weekly average of ECM yield during the whole experiment was affected by the interaction of treatment and week of lactation ($P < 0.01$; Supplemental Figure S3, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a); CLA and HSO groups had lower ECM yield compared with CON throughout the study. The HSO group had consistently lower ECM yield than CON whereas CLA became closer to CON over time. Weekly milk fat content and yield (Supplemental Figures S4 and S5, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a) were the highest for CON, intermediate

for the CLA group, and the lowest for the HSO group throughout the 16 wk ($P < 0.01$). However, milk fat concentration dropped quicker for HSO compared with the CLA diet over wk 2 and 3 of lactation whereas milk fat yield was more stable with HSO compared with other diets throughout the 16 wk ($P < 0.01$ for interaction between treatment and time).

Energy and Nitrogen Utilization and BW

The proportion of GE intake excreted as feces and methane was lower ($P < 0.01$) for cows fed HSO compared with the CON and CLA groups throughout the
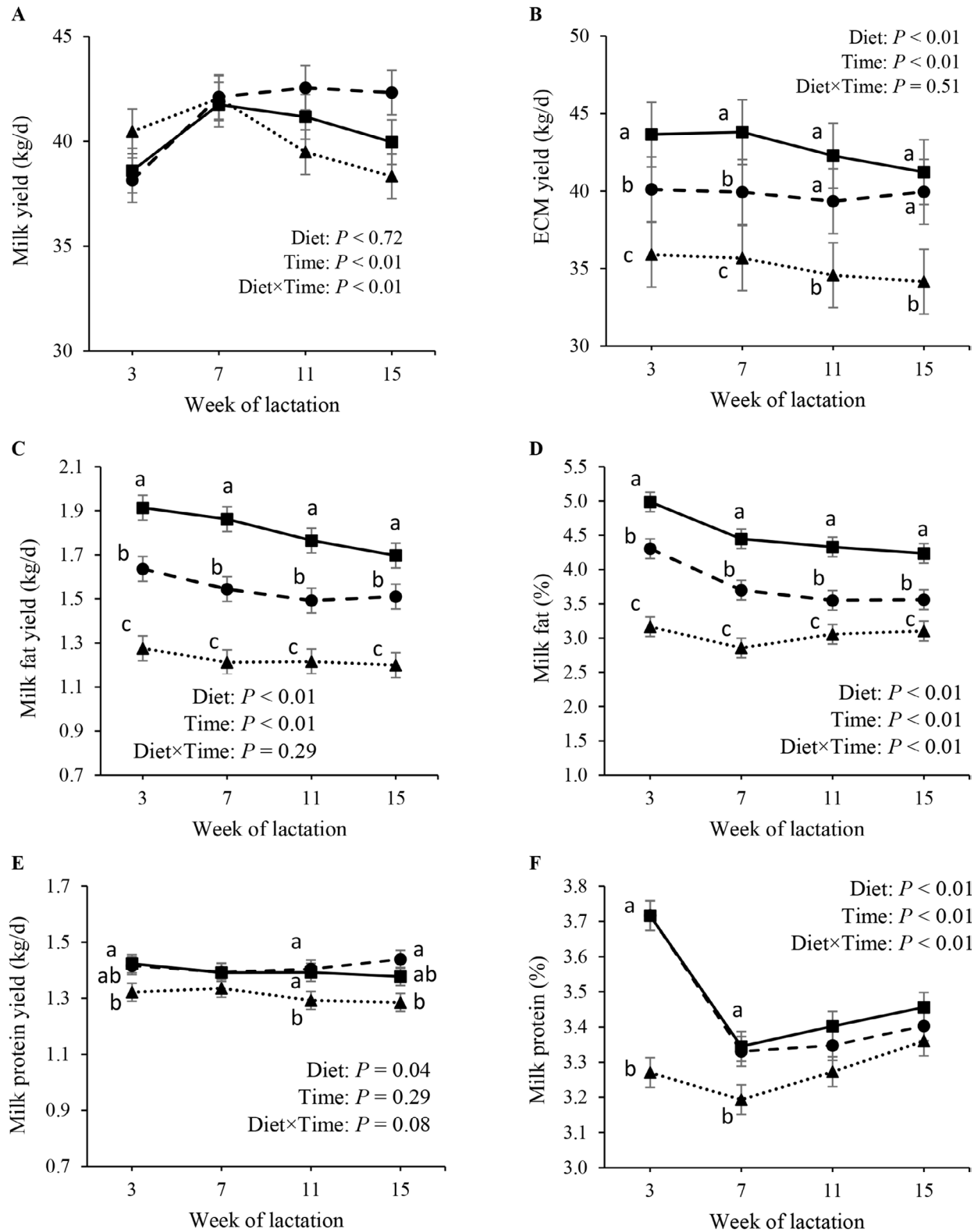
Table 2. Effect of dietary CLA supplement and higher starch and oil-containing diet (HSO) on nutrients intake of dairy cows fed grass silage-based diets during the first 112 d of lactation

Item	CON	CLA	HSO	SEM	Diet	Time	Diet × Time
	Diet[1]				P-value		
Intake, kg/d							
DM							
Mean	26.8[a]	26.6[a]	21.6[b]	0.40	<0.01		
Wk 3	23.7[C,a]	24.1[B,a]	20.2[B,b]	0.62	<0.01	<0.01	0.068
Wk 7	26.6[B,a]	26.6[A,a]	21.8[A,b]				
Wk 11	28.8[A,a]	27.6[A,a]	21.8[A,b]				
Wk 15	27.9[AB,a]	28.1[AB,a]	22.5[A,b]				
OM							
Mean	24.7[a]	24.6[a]	20.1[b]	0.37	<0.01		
Wk 3	21.9[C,a]	22.3[C,a]	18.8[B,b]	0.46	<0.01	<0.01	<0.01
Wk 7	24.6[B,a]	24.6[B,a]	20.3[A,b]				
Wk 11	26.7[A,a]	25.6[AB,a]	20.3[A,b]				
Wk 15	25.8[AB,a]	26.0[A,a]	20.9[A,b]				
CP							
Mean	4.24[a]	4.21[a]	3.37[b]	0.064	<0.01		
Wk 3	3.74[C,a]	3.80[B,a]	3.16[B,b]	0.096	<0.01	<0.01	0.04
Wk 7	4.23[B,a]	4.22[A,a]	3.40[AB,b]				
Wk 11	4.56[A,a]	4.38[A,a]	3.40[AB,b]				
Wk 15	4.42[AB,a]	4.44[A,a]	3.52[A,b]				
NDF							
Mean	10.2[a]	10.1[a]	7.21[b]	0.14	<0.01		
Wk 3	9.02[C,a]	9.14[B,a]	6.77[B,b]	0.23	<0.01	<0.01	0.03
Wk 7	10.2[B,a]	10.2[A,a]	7.31[AB,b]				
Wk 11	11.0[A,a]	10.5[A,a]	7.28[AB,b]				
Wk 15	10.6[AB,a]	10.5[A,a]	7.45[A,b]				
Starch							
Mean	4.38	4.36	4.35	0.070	0.95		
Wk 3	3.85[C]	3.93[B]	3.99[B]	0.11	0.95	<0.01	0.28
Wk 7	4.33[B]	4.33[A]	4.41[A]				
Wk 11	4.76[A,a]	4.57[A,ab]	4.43[A,b]				
Wk 15	4.59[AB]	4.62[A]	4.57[A]				
Gross energy, Mcal/d							
Mean	115[a]	115[a]	97.0[b]	1.8	<0.01		
Wk 3	102[C,a]	105[C,a]	90.8[B,b]	2.7	<0.01	<0.01	0.075
Wk 7	115[B,a]	115[B,a]	98.5[A,b]				
Wk 11	124[A,a]	120[A,a]	97.8[A,b]				
Wk 15	120[A,a]	121[A,a]	101[A,b]				

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[A–C]Within a column, means without a common superscript differ ($P < 0.05$).

[1]CON refers to a low starch (16.4% DM) grass silage-based diet containing 55:45 forage-to-concentrate ratio on DM basis (n = 13); CLA refers to the control diet supplemented with 100–150 g/d of rumen-protected CLA (n = 9); HSO = a higher starch (20.2% DM) diet containing 4.0% mixture of UFA (sunflower and fish oils 2:1 wt/wt; n = 11).

Figure 1. Effect of dietary CLA supplement and higher starch and oil-containing diet (HSO) on milk yield (A), ECM yield (B), milk fat yield (C), milk fat concentration (D), milk protein yield (E), milk protein concentration (F) of dairy cows during the first 112 d of lactation. Control = control diet (n = 13, ■ and solid line); CLA = CLA-supplemented diet (n = 9, ● and dashed line); HSO = higher starch diet supplemented with sunflower and fish oil (2:1) (n = 11, ▲ and dotted line). Different letters indicate the significant difference ($P < 0.05$) between groups at different time points. Error bars represent SEM.

experiment (Table 3). Energy required for milk production as a proportion of ME intake was lower for both the CLA and HSO groups compared with the CON group at wk 3 whereas at wk 7, only CLA group had a lower value compared with CON, and at wk 11 and 15 there was no difference between the treatments. Cows fed HSO showed a greater ($P < 0.01$) metabolic heat production as a proportion of energy intake or as ME or GE intake ($P < 0.05$) compared with the CON and CLA groups during wk 3, 7, 11, and 15 of lactation.

Table 3. Effect of dietary CLA supplement and higher starch and oil-containing diet (HSO) on energy partitioning of dairy cows fed grass silage-based diets during the first 112 d of lactation

Item[2]	Diet[1]			SEM	*P*-value		
	CON	CLA	HSO		Diet	Time	Diet × Time
Feces, Mcal/100 Mcal of energy intake							
Mean	28.6[a]	28.5[a]	25.3[b]	0.50	<0.01		
Wk 3	28.5[A,a]	28.5[a]	24.7[b]	0.74	<0.01	0.19	0.62
Wk 7	29.1[A,a]	28.8[a]	25.3[b]				
Wk 11	27.3[B,ab]	28.0[a]	25.5[b]				
Wk 15	29.4[A,a]	28.8[a]	25.6[b]				
Urine, Mcal/100 Mcal of energy intake							
Mean	3.76	3.78	4.01	0.001	0.18		
Wk 3	3.79[b]	3.98[ab]	4.25[A,a]	0.001	0.18	0.050	0.43
Wk 7	3.74	3.62	3.91[B]				
Wk 11	3.69	3.79	4.05[B]				
Wk 15	3.83	3.73	3.83[B]				
Methane, Mcal/100 Mcal of energy intake							
Mean	6.31[a]	6.40[a]	5.52[b]	0.095	<0.01		
Wk 3	6.26[a]	6.51[a]	5.39[b]	0.116	<0.01	0.71	0.018
Wk 7	6.36[a]	6.53[a]	5.46[b]				
Wk 11	6.25[a]	6.30[a]	5.72[b]				
Wk 15	6.37[a]	6.26[a]	5.52[b]				
Milk, Mcal/100 Mcal of energy intake							
Mean	28.3[a]	26.5[b]	27.4[ab]	0.49	0.081		
Wk 3	32.4[A,a]	29.1[A,b]	29.7[A,b]	0.72	0.081	<0.01	0.069
Wk 7	28.8[B,a]	26.7[B,b]	27.7[B,ab]				
Wk 11	25.8[C]	25.3[B]	26.7[BC]				
Wk 15	26.0[C]	24.9[B]	25.6[C]				
Heat, Mcal/100 Mcal of energy intake							
Mean	33.1[c]	34.0[b]	37.4[a]	0.22	<0.01		
Wk 3	34.6[A,b]	35.1[A,b]	38.8[A,a]	0.41	<0.01	<0.01	0.86
Wk 7	33.4[B,b]	34.3[AB,b]	37.8[AB,a]				
Wk 11	32.1[C,c]	33.4[B,b]	37.0[B,a]				
Wk 15	32.3[BC,b]	33.2[B,b]	36.1[B,a]				
MEI/GEI, Mcal/100 Mcal							
Mean	61.4[b]	61.3[b]	65.2[a]	0.52	<0.01		
Wk 3	61.4[AB,b]	61.0[b]	65.7[a]	0.80	<0.01	0.41	0.44
Wk 7	60.9[B,b]	61.1[b]	65.3[a]				
Wk 11	62.8[A,ab]	61.9[b]	64.8[a]				
Wk 15	60.4[B,b]	61.3[b]	65.0[a]				
Milk energy/MEI, Mcal/100 Mcal							
Mean	43.1[a]	40.4[ab]	39.7[b]	0.86	0.019		
Wk 3	49.6[A,a]	44.9[A,b]	42.8[A,b]	1.40	0.019	<0.01	0.13
Wk 7	44.2[B]	40.9[B]	39.9[B]				
Wk 11	38.5[C]	38.1[B]	38.8[B]				
Wk 15	40.2[C]	37.8[B]	37.2[B]				
Energy balance, Mcal/d							
Mean	0.80	1.79	0.81	0.856	0.71		
Wk 3	−5.28[C]	−3.04[B]	−2.15[B]	1.528	0.71	<0.01	0.15
Wk 7	−1.09[B]	1.36[B]	0.12[AB]				
Wk 11	6.33[A,a]	4.64[A,ab]	1.35[AB,b]				
Wk 15	3.23[A]	4.18[A]	3.94[A]				

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[A–C]Within a column, means without a common superscript differ ($P < 0.05$).

[1]CON refers to a low starch (16.4% DM) grass silage-based diet containing 55:45 forage-to-concentrate ratio on DM basis (n = 13); CLA refers to the control diet supplemented with 100–150 g/d of rumen-protected CLA (n = 9); HSO = a higher starch (20.2% DM) diet containing 4.0% mixture of UFA (sunflower and fish oils 2:1 wt/wt; n = 11).

[2]MEI = metabolizable energy intake; GEI = gross energy intake.

Milk energy as a proportion of ME intake was lower ($P < 0.05$) for CLA and HSO compared with CON during wk 3. Energy balance was negative for all treatments at wk 3, improved at wk 7 and was positive for all diets afterward ($P < 0.01$; Figure 2). Treatments did not significantly affect EB in dairy cows, however, feeding the CLA or HSO diets had numerical positive effects on this variable at the first weeks of lactation (both CLA and HSO groups had higher numerical EB at wk 3 and 7 compared with CON), whereas at wk 11, the HSO treatment had lower EB compared with CON (1.35 vs. 6.33 Mcal/d). At wk 15, EB did not differ between diets. Feeding the HSO diet decreased N intake ($P < 0.01$) compared with the other 2 treatments (Supplemental Table S2, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a). Furthermore, feeding HSO increased ($P < 0.01$) efficiency of dietary N utilization, expressed as the ratio of milk N to N intake, compared with CON and CLA diets during all measurement times, whereas the proportion of dietary N excreted in feces decreased ($P = 0.08$) when HSO was fed at wk 15 compared with the CON and CLA groups. The BW was greater for CLA cows compared with the CON during wk 11 and 15, whereas the CLA and HSO cows were not significantly different. Cows in the CLA and HSO groups had less BW loss at wk 7 and better weight gain at wk 11 compared with the CON group (Supplemental Table S3, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a).
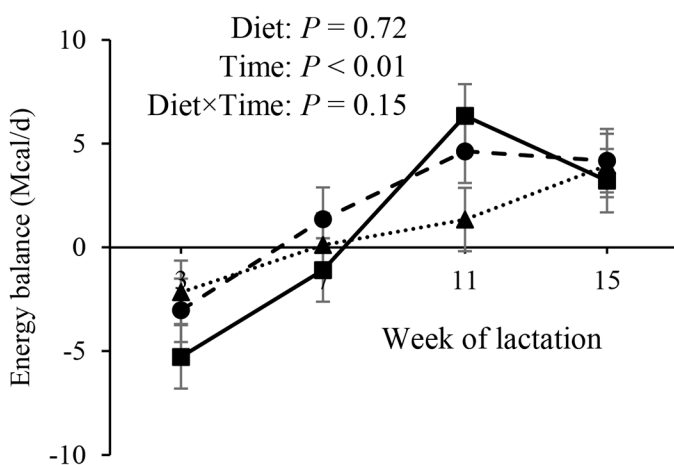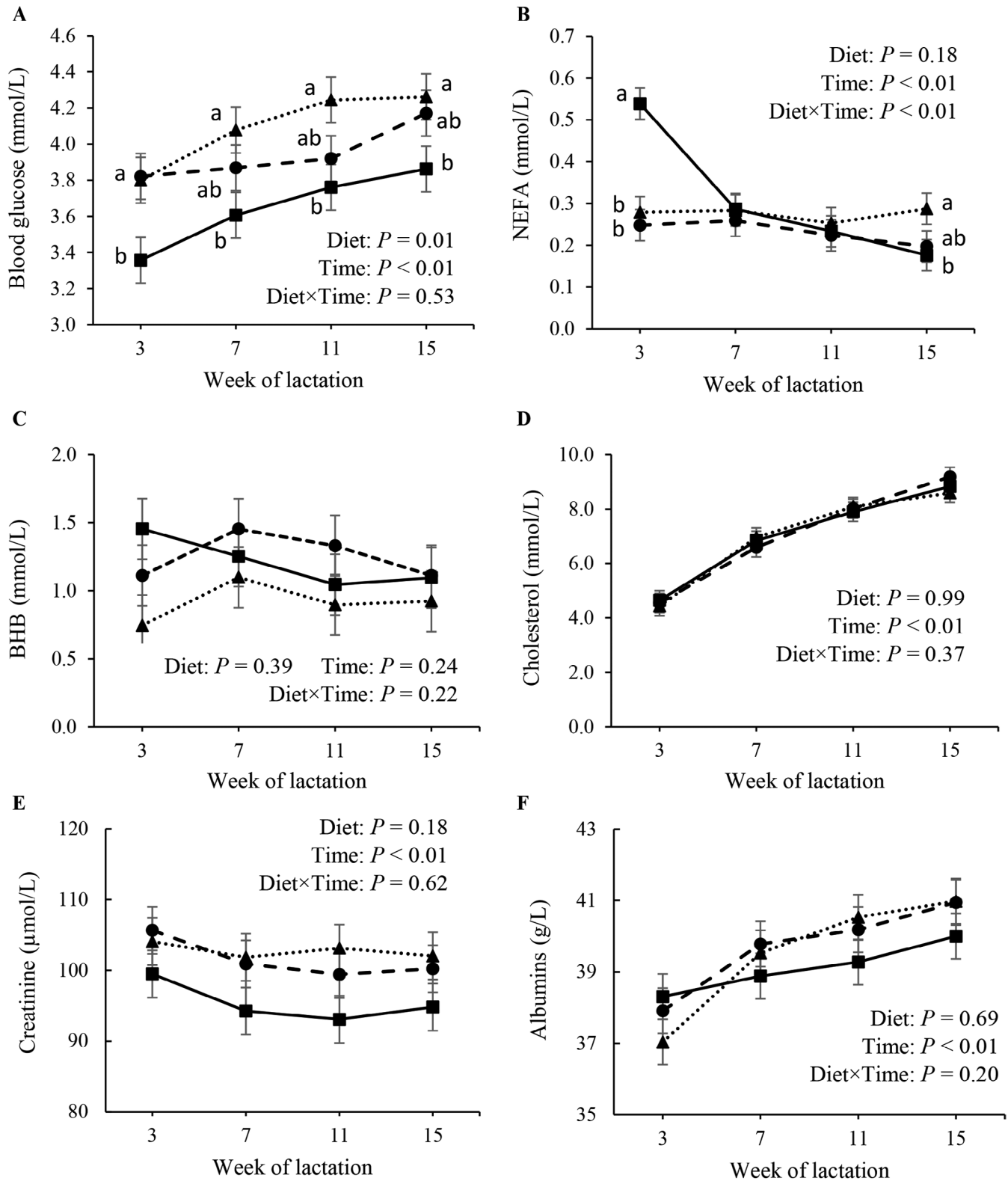

Figure 2. Effect of dietary CLA supplement and higher starch and oil-containing diet (HSO) on energy balance of dairy cows during the first 16 wk of lactation. Control = control diet (n = 13, ■ and solid line); CLA = CLA-supplemented diet (n = 9, ● and dashed line); HSO = higher starch diet supplemented with sunflower and fish oil (2:1) (n = 11, ▲ and dotted line). Error bars represent SEM.

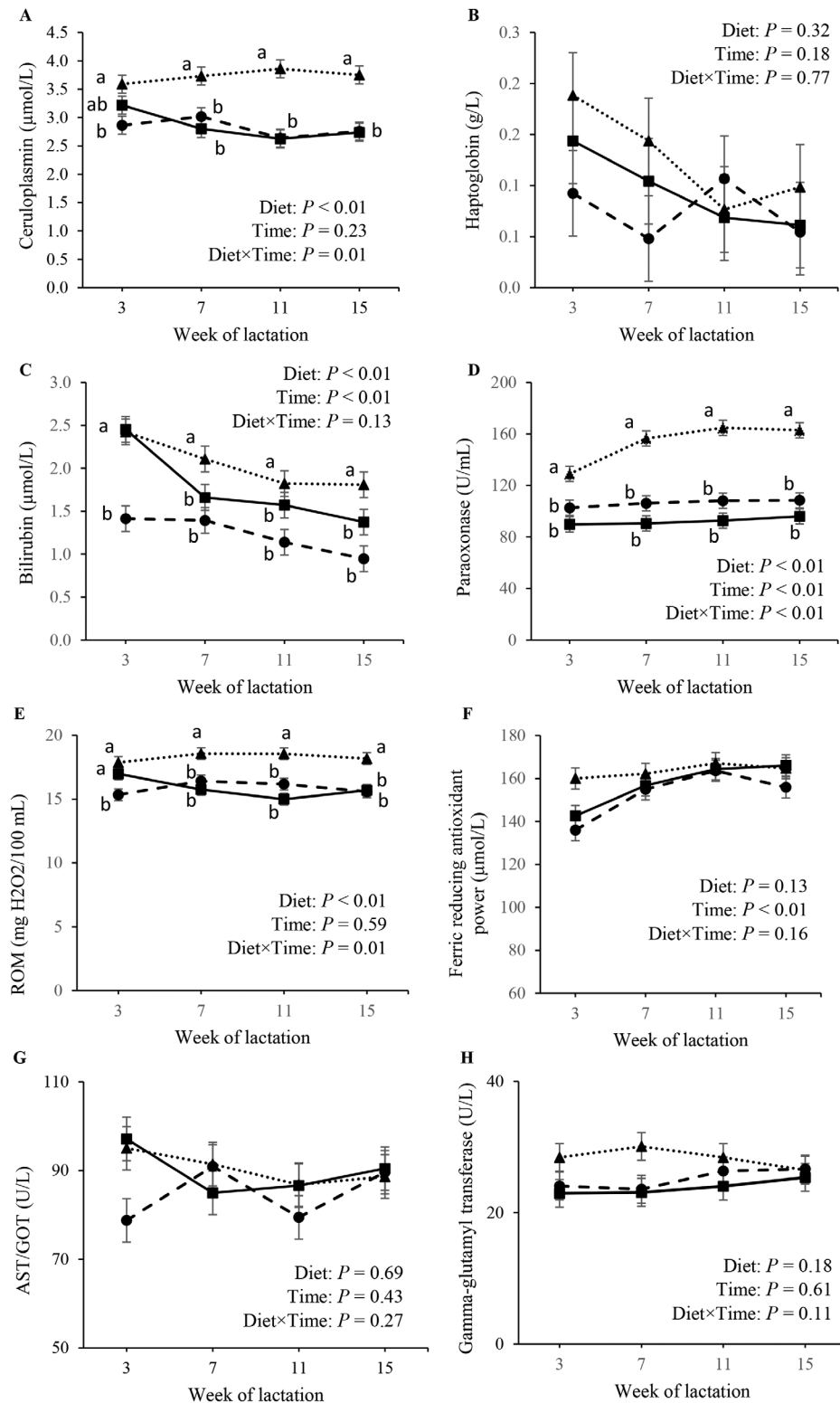## Blood Metabolites and Inflammation and Oxidative Status

Effects of dietary treatments on blood metabolites, hormones, and inflammation and oxidative status are presented in Figure 3, Supplemental Table S4 (https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a) and Figure 4, respectively. During the experiment, most of the metabolites were affected by time. Plasma concentrations of glucose, cholesterol, albumin, triglycerides, FRAP, IGF-I, and leptin concentrations increased ($P \leq 0.014$), and total bilirubin, creatinine, and somatotropin concentrations decreased ($P \leq 0.043$) over time regardless of dietary treatments. Plasma concentrations of cholesterol, haptoglobin, IGF-I, and leptin were not different ($P \geq 0.30$) between treatments during the experiment. An interaction between treatment and time was observed in blood concentration of NEFA ($P < 0.01$; Figure 3B). At wk 3, we observed lower blood NEFA concentration for the CLA and HSO diets compared with the CON diet, whereas this variable was higher in the HSO group compared with CON at wk 15. The NEFA concentration decreased gradually over time for the CON diet whereas there was no significant trend in time for CLA and HSO treatments. In contrast, blood BHB concentration did not differ among treatments.

Interaction trend was observed for the blood urea concentration as the HSO diet had lower value ($P < 0.05$) compared with CON at wk 11 and 15. In the CON cows, blood urea concentration increased gradually over time and plasma triglycerides concentration increased ($P < 0.01$) as lactation progressed in all treatments (Supplemental Figure S6, https://doi.org/10.17632/fckmgfwpnp.1, Bayat et al., 2022a). In addition, higher ($P = 0.011$) blood glucose concentrations were observed in the HSO group compared with the CON group in all measurement times, whereas cows fed the CLA diet had higher blood glucose concentration compared with the CON group only at wk 3 (Figure 3). Blood albumin slightly increased over time ($P < 0.01$) in the CLA and HSO cows but was not affected by treatments. Plasma creatinine decreased ($P < 0.01$) over time for all treatments (Figure 3). Serum IGF-I, insulin, leptin, and glucagon concentrations were not affected ($P \geq 0.30$) by the treatments (Supplemental Table S4, Bayat et al., 2022a). However, IGF-I concentration increased ($P < 0.01$) by feeding the CON and CLA diets from wk 7 to 15 of lactation, whereas somatotropin concentration decreased in CON cows from wk 7 to 15 of lactation ($P < 0.04$).

Concentration of ceruloplasmin (a positive APP) was higher for the HSO compared with the CON and CLA groups throughout the experiment (Figure 4). It

Figure 3. Effect of dietary CLA supplement and higher starch and oil-containing diet (HSO) on plasma metabolite concentrations of (A) blood glucose, (B) nonesterified fatty acids (NEFA), (C) BHB, (D) cholesterol, (E) creatinine, and (F) albumin of dairy cows during the first 16 wk of lactation. Control = control diet (n = 13, ■ and solid line); CLA = CLA-supplemented diet (n = 9, ● and dashed line); HSO = higher starch diet supplemented with sunflower and fish oil (2:1) (n = 11, ▲ and dotted line). Different letters indicate the significant difference ($P < 0.05$) between groups at different time points. Error bars represent SEM.

Figure 4. Effect of dietary CLA supplement and higher starch and oil-containing diet (HSO) on inflammation and oxidative status: (A) ceruloplasmin, (B) haptoglobin, (C) bilirubin, (D) paraoxonase, (E) reactive oxygen metabolites (ROM), (F) ferric reducing antioxidant power, (G) aspartate aminotransferase or glutamic-oxaloacetic transaminase (AST/GOT), and (H) gamma-glutamyl transferase of dairy cows during the first 16 wk of lactation. Control = control diet (n = 13, ■ and solid line); CLA = CLA-supplemented diet (n = 9, ● and dashed line); HSO = higher starch diet supplemented with sunflower and fish oil (2:1) (n = 11, ▲ and dotted line). Different letters indicate the significant difference ($P < 0.05$) between groups at different time points. Error bars represent SEM.

decreased over time for CON and remained unchanged for CLA and HSO groups ($P < 0.05$ for interaction between treatment and times). Treatments did not affect ($P \geq 0.13$) concentrations of haptoglobin (another positive APP) and aspartate aminotransferase or glutamic-oxaloacetic transaminase in all measurement times. Plasma paraoxonase concentration (a negative APP) was greater ($P < 0.01$) for HSO than CON and CLA diets and increased rapidly from wk 3 to 7 of lactation for HSO ($P < 0.01$ for the interaction of treatment and time; Figure 4). Feeding the CLA diet decreased total bilirubin concentration compared with CON and HSO at wk 3, whereas it was higher for HSO in comparison to other groups during wk 7 to 15 of lactation ($P < 0.01$). Overall, plasma ROM was lower ($P < 0.01$) for the CLA and CON groups compared with HSO group ($P < 0.01$). At wk 3, ROM concentration was similar in CON to HSO, with a rapid decrease in the CON group from wk 3 to wk 11 but was stable for the CLA and HSO groups over time ($P < 0.01$; for the interaction of treatment and time). The effect of time was significant for FRAP as it increased ($P < 0.01$) during wk 3 to 15 of lactation in the CON and CLA cows.

DISCUSSION

In early lactation, cows are usually in negative EB. To alleviate this condition, a milk fat-depressing diet can be fed as a possible nutritional tool to prevent metabolic disorders and enhance overall health and lactation performance. Milk fat represents an important energy excretion component for lactating cows. The MFD does not only result in spared energy that can repartition to adipose tissue, but it may also lead to reduced energy intake (Harvatine et al., 2009). However, reducing milk fat yield by dietary supplementation of CLA isomers or alternatively feeding diets containing higher dietary starch content plus PUFA sources may result in increased milk yield causing no effect on energy output (Griinari et al., 1998; Medeiros et al., 2010; reduction in milk fat yield by 35 and 20%, respectively) or improvement in EB (Odens et al., 2007; von Soosten et al., 2011). There has been an interest in using *trans*-10,*cis*-12 CLA to modulate energy metabolism of dairy cows, with the rationale that decreasing fat output in milk would improve EB (Bauman et al., 2008). Our goal was to evaluate the energy utilization in early lactating cows fed a diet which can induce MFD (higher dietary starch content plus PUFA-rich lipid supplement) compared with a low dose of rumen-protected CLA supplement (0.39–0.56% of diet DM) and to document whether the responses detected in this study result from changes in inflammo-metabolic status or

nutrient utilization by postpartum diet-induced MFD or CLA-specific.

Nutrient Intake

Intakes of DM and GE decreased markedly (-19 and -16%, respectively) in cows fed HSO compared with the CON diet. Energy requirement is one the most important factors for regulating feed intake (Forbes, 2005), and this might explain the reduction in DMI especially with the HSO diet as a reflection of lower milk energy output as will be discussed later. In a meta-analysis, Rabiee et al. (2012) reported that DMI was decreased by all dietary lipid supplementations (on average -0.88 kg of DM/d). In the current study, the DMI reduced strongly by HSO compared with CON (-5.2 kg/d on average for all measurement times) despite the level of supplemented lipid in the HSO diet not exceeding the upper limit of 3 to 4% DM (equal to 6–7% DM total lipids in the diet) recommended by NRC (2001) to avoid impairing effects on DMI. Detrimental effects of plant oils on DMI might have arisen from effect of oils on the palatability of the diet or by altering release of gut hormones or lipid oxidation in the liver (Kuhla et al., 2016). In addition, adverse effects on ruminal fermentation and rumen epithelium functionality, such as reducing cellulolytic activity and increasing rumen permeability to absorption of immunogenic molecules such as lipopolysaccharides (Minuti et al., 2015), might contribute to the lower DMI. The remarkable decrease in average DMI of HSO cows may be due to potent hypophagic effect of PUFA sources (~870 g/d) especially from fish oil on DMI in the early postpartum period, as described by Kuhla et al. (2016). Our data do not reveal the mechanism causing the decreased DMI; however, fish oil even in low amounts (1.5% of diet DM in combination with 3% sunflower oil) has shown a strong adverse effect on DMI, which agrees with the study of Shingfield et al. (2006) where the feed intake was reduced by 21%.

On the other hand, DMI was not affected by the CLA supplement in the present study. Previously, a decrease (Schäfers et al., 2017), increases (de Veth et al., 2005; Castañeda-Gutiérrez et al., 2005) or no changes (Odens et al., 2007; von Soosten et al., 2011) in DMI by dietary CLA supplementation under different stages of lactation, feeding, and dosing conditions have been reported. In a review, Bauman et al. (2008) reported that in the periods of inadequate energy intake, CLA-induced MFD has marginal or no detectable effects on DMI which is in line with our results. Overall, CLA effects are also associated with other important production parameters (e.g., nutrients intake, composition of

diets, stage of lactation, and BCS), which explains the large variability among CLA studies.

Milk Production and Composition

The lack of difference in milk yield but lower milk fat yield with the CLA and HSO diets are consistent with the hypothesis to reduce milk fat content without compromising milk yield (Schäfers et al., 2017, Ventto et al., 2017). Using a meta-analysis of 14 studies, Harvatine et al. (2009) reported no change in milk yield of dairy cows abomasally infused with CLA, which is in line with our results. A concomitant increase in milk yield and decrease in milk fat content was reported in studies where cows were fed CLA supplements in early lactation (de Veth et al., 2005; Moallem et al., 2010) or on pasture-based systems (Medeiros et al., 2010). However, in our study feeding rumen-protected CLA to early-lactation cows did not change milk yield although milk fat content and yield decreased. Lack of a difference in milk yield despite the lower DMI with the HSO compared with the CON and CLA cows, can be attributed to the higher total-tract digestibility of OM and GE (Hristov et al., 2004).

The milk fat content responses to CLA and HSO diets were different and HSO showed stronger and faster inhibitory effect on milk fat yield when compared with the CLA group (−32.2 vs. −16.0%). Harvatine and Bauman (2006) observed that milk fat content decreased 30% with low-forage, high oil diet (the mixture of 1.5% fish oil and 3.0% soybean oil) and 24% with the 3-d infusion of trans-10,cis-12 CLA in mid-lactation cows. In the present study, 100 g of encapsulated CLA supplement provided 20 g/d of total CLA, containing 10 g of trans-10,cis-12 18:2 isomer, which is higher than the range used by others to achieve MFD (≥4.6 g of trans-10,cis-12 CLA per day). In a study from our team (Kairenius, 2020), MFD was induced when 200 g of fish oil, or the mixtures of fish oil and sunflower oil (200 + 500 g/d) or fish oil and linseed oil (200 + 500 g/d) were supplemented to grass silage-based diets, which decreased milk fat content by −11.8, −19.3, and −27.7%, respectively. Further, Shingfield et al. (2006) reported a reduction in milk fat yield by 37% in dairy cows fed diet containing 4.5% sunflower oil and fish oil mixture, and 16% starch. Unprotected rumen PUFA source used in the present study contained mostly linoleic acid and n-3 very long-chain PUFA, therefore we anticipated that a substantial portion of the PUFA is dissociated in the rumen and undergo BH. In addition, greater dietary starch concentration (provided partly from wheat grain) likely affect more acidic rumen environment which would have helped to create a scenario

leading to increased formation of FA isomers linked to MFD (Kleen et al., 2003; Ventto et al., 2017).

The mechanism of trans-10,cis-12 CLA-induced MFD has been well characterized (Baumgard et al., 2002; Harvatine and Bauman, 2006). However, recently the potential involvement of ruminal trans-10,cis-15 18:2 formation in MFD with combination of dietary fish oil and plant oils (Kairenius, 2020) or sunflower oil (Ventto et al., 2017) has been postulated. Briefly, transcription of key mammary lipogenic genes is coordinately downregulated in mammary gland (Bauman et al., 2011). Although changes in mammary tissue were not directly analyzed, the current study provides insight into changes in pathways involved in milk fat synthesis.

Earlier studies have reported that dietary mixture of fish and sunflower oil (Shingfield et al., 2006) often decrease milk protein content. Wu and Huber (1994) identified negative effects of fat feeding on milk protein percentage and speculated that decreased glucose availability, development of insulin resistance and increased milk yield may be involved in the decreased protein percentage. However, dietary energy intake of the dairy cow is the primary nutritional factor affecting milk protein content (Lock and Shingfield, 2004), suggesting that the effects of PUFA on DMI (21.6 vs. 26.8 kg/d for the HSO and CON groups, respectively) were largely responsible for the reductions in milk protein content in HSO cows.

Energy Metabolism

It was expected that reducing milk energy output may improve the EB of the cows. In the present study, decreased milk energy output was due mainly to a dramatic decrease in the average of milk fat content from 4.5 to 3.78 and 3.05% with the CLA and HSO groups, respectively. We observed a lower milk energy output as a proportion of ME intake (rather equal to higher milk production efficiency) with the CLA and HSO groups compared with the CON group at wk 3, whereas there was no difference among the treatments for this variable at wk 7, 11, and 15. As milk energy output is one of the main fractions of energy output in a high producing dairy cow (28.3% of energy intake for CON), it is expected that lowering milk energy output improves EB of the cows allowing repartitioning of metabolizable energy to body reserves. As indicated in the companion paper (Qin et al., 2018), both MFD-inducing strategies decreased the mobilization of body fat from subcutaneous adipose tissue in early lactation based on the reduced transcription of lipolytic genes. The CLA diet upregulated the transcription of various genes involved in insulin signaling, inflammatory responses,

and ceramide metabolism and therefore, might have regulated insulin sensitivity in adipose tissue. However, no changes were observed in these pathways in cows receiving the higher starch diet containing oil indicating that CLA supplementation and the diet-induced MFD differ in their mode of actions. Our results suggest that when the HSO diet is fed, EB is likely improved, but the effect is masked by the depressed feed intake.

In the present study, the CLA supplement and the HSO induced only numerical improvement in EB in wk 3 and 7 postpartum despite significant decreases in milk energy output. However, lower plasma NEFA and BHB at wk 3 in the CLA and HSO cows suggest a lower mobilization from adipose tissue and lower ketogenesis in the liver, indicating a better energy utilization efficiency. Improved functionality of the liver is also confirmed by the lower concentrations of bilirubin, the higher blood glucose at wk 3 and the lower oxidative stress (lower ROM) in the CLA group compared with the CON cows. Our findings are similar to those reported by Castañeda-Gutiérrez et al. (2005) who showed at a high dose (18 g/d of *trans*-10,*cis*-12 CLA), the CLA-induced MFD decreased milk energy output without improvement in net EB or decrease in the use of body fat as evaluated by changes in BW or BCS.

Compared with the CON diet, feeding the HSO diet reduced methane emissions (−28.7% as g/d or −18.3% as a proportion of energy intake on average). The decrease in methane emissions in the HSO group compared with CON may be substantially explained by supplemented PUFA and increased starch concentration from 16.4 to 20.2% of DM. Dietary fat supplementation has been shown in many studies to reduce methane emissions. Consistent with our finding, Woodward et al. (2006) observed a 27% reduction in methane yield by feeding 3.75% DM of mixture of sunflower and fish oil; however, they did not observe any negative effect on feed intake. Polyunsaturated FA inhibit methanogenesis by reducing the metabolic activity and number of ruminal methanogens (Lillis et al., 2011) and protozoa, decreasing the amount of carbohydrate fermented in the rumen, and through BH of UFA.

Plasma Metabolites, Hormones, and Inflammation Markers

The NEFA is an important source for mammary fat synthesis and for energy production in the liver (Bauman and Griinari, 2003), and can be used as an indicator of lipid mobilization and EB in dairy cows. The lower blood NEFA concentration in the CLA and HSO groups at wk 3 indicates less lipid mobilization and further supports the improved EB in these groups compared with the CON group. Our results are in line

with previous studies in which plasma NEFA levels decreased by dietary supplement of CLA isomers at early lactation (Odens et al., 2007; Trevisi et al., 2008). Usually when EB is negative, plasma BHB is higher indicating the incomplete β-oxidation of mobilized body fat. The variations of plasma BHB in this study agree with those of NEFA and support the improvement of the efficiency of the liver in the oxidation of NEFA in cows fed CLA and HSO compared with those fed CON.

The MFD induced by the CLA and HSO diets was accompanied by increases in plasma glucose concentration at wk 3 and across the entire experiment especially for the HSO diet. These results are consistent with previous results from cows fed CLA supplement in the first 7 wk of lactation (Odens et al., 2007; Hötger et al., 2013) and from those fed n-3 PUFA-rich fish oil and linseed (Ballou et al., 2009). Hötger et al. (2013) demonstrated that *trans*-10,*cis*-12 CLA reduced endogenous glucose production and at the same time reduced the glucose demand in the tissues, eventually leading to increased plasma glucose concentration. Otherwise, higher plasma glucose in the HSO cows can be also supported by a condition of inflammation, demonstrated by the higher level of plasma ceruloplasmin. This higher level could be due to more rumen fermentable carbohydrates and the presence of lipid peroxides in HSO cows (Minuti et al., 2015).

Interestingly, compared with the CON and CLA groups, the HSO-fed cows had higher plasma ceruloplasmin, paraoxonase, and total bilirubin concentrations in the entire experiment. Moreover, the HSO cows showed the highest levels of ROM with similar FRAP concentrations (at wk 3 postpartum FRAP was higher compared with other groups). The increased inflammatory and oxidative stress state with no translation into reduced milk production in the HSO cows can be attributed to the negative effects of free PUFA on rumen epithelium permeability that leads to greater absorption of immunogenic molecules (i.e., histamine and endotoxins), activates an immunogenic response in forestomach, and spikes APP concentration (Trevisi et al., 2014a; Minuti et al., 2015). The activity of paraoxonase, an enzyme capable of hydrolyzing lipid peroxides, was particularly high in the HSO in comparison to the other groups and previous experiments (Bionaz et al., 2007; Trevisi et al., 2012) and could represent an adaptive mechanism to contrast abundant lipid peroxides, likely supplied with the diet. Nevertheless, further studies are necessary to clarify this specific aspect, never signaled previously.

Inflammatory responses, in term of positive APP, were not affected by the CLA treatment. However, the CLA cows had the lowest concentrations of total bilirubin and oxidative stress status (i.e., ROM) which

suggest less inflammatory challenges in the CLA group compared with other groups during the experiment. Moreover, the HSO cows showed the highest values of ROM concentration in plasma without a correspondent raise of antioxidants, which advocates a condition of oxidative stress more severe than other groups, likely due to the higher intake of lipid peroxides with the diet. Our findings support previous results of Trevisi et al. (2008) which reported higher plasma levels of some negative APP (cholesterol and albumin) and a better oxidative status (similar ROM, but higher levels of thiol groups) in the first month of lactation in dairy cows when fed with a CLA supplement. As far as blood parameters are concerned, in the present study, the HSO cows experienced increased inflammatory and oxidative stress state whereas the CLA cows experienced milder inflammatory challenge compared with other groups during the experiment.

CONCLUSIONS

Both dietary supplemented rumen-protected CLA and HSO diets reduced milk fat production without compromising milk yield. The HSO diet had a negative effect on feed and energy intakes, whereas it resulted in higher ME/GE intake compared with the CON and CLA groups. Energy balance was numerically higher for the CLA and HSO groups at wk 3 and 7 postpartum. All cows experienced a degree of apparent inflammation after calving, but cows supplemented with CLA had lower inflammation and oxidative stress after calving compared with CON and HSO. These results suggest that MFD induced by either the HSO diet or CLA supplementation had moderate positive effects on EB at the first weeks of lactation. The negative effect of HSO on feed intake and the indication of increased inflammatory and oxidative stress warrant further studies before the HSO feeding strategy can be supported as an alternative to improve EB in early lactation.

ACKNOWLEDGMENTS

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