J. Dairy Sci. 85:580–594

Supplementation with Partially Hydrogenated Oil in Grazing Dairy Cows in Early Lactation

G. F. Schroeder,* G. A. Gagliostro,† D. Becu-Villalobos,‡ and I. Lacau-Mengido‡
*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
†National Institute of Agricultural Technology (INTA),
Experimental Station of Balcarce, CC 276 (7620), Balcarce, Argentina
‡Instituto de Biología y Medicina Experimental (CONICET), V. Obligado 2490, Buenos Aires, Argentina

ABSTRACT

Effects of partially hydrogenated oil on performance, loss of body weight and body condition score, and blood metabolite and hormone concentrations were evaluated in 37 multiparous Holstein cows in grazing conditions during the first 100 d of lactation. Six additional Holstein cows, each fitted with a ruminal cannula, were allocated to a replicated 3 × 3 Latin square to evaluate effects of supplemental fat on rumen environment and pasture digestion. All cows grazed mixed pastures based on alfalfa (*Medicago sativa*) and orchardgrass (*Dactylis glomerata* L.) and received 5.4 kg/d of a basal concentrate to which 0, 0.5, or 1 kg/cow per day of partially hydrogenated oil (melting point 58 to 60°C) containing 30.3, 34.9, 21.8, and 3.3% of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$, respectively, was added. Feeding 1 kg/d of supplemental fat increased fat-corrected milk from 23.4 to 26.3 kg/d, milk fat content from 3.44 to 3.78%, and milk fat yield from 0.87 to 1.03 kg/d compared to control. Milk protein percentage and yield were not affected. Cows fed 1 kg/d of fat increased the content and yield of $C_{16:0}$ and $C_{18:0}$ in milk compared with cows fed no added oil. Dry matter intake (DMI) from pasture decreased from 17.8 kg/d for control cows to 13.6 kg/d for cows fed 1 kg of oil, whereas DMI from concentrate was higher for cows fed 1 kg/d of fat (6.0 kg/d) than for controls (5.2 kg/d). Supplemental fat did not affect total dry matter or estimated energy intake and did not change losses of body weight or body condition scores. Plasma concentrations of nonesterified fatty acids, insulin, somatotrophin, and insulin-like growth factor-I did not differ among treatments. Concentration of plasma triglycerides was lowered from 318.5 to 271.2 mg/dl, whereas plasma cholesterol was elevated from 185.0 to 235.8 mg/dl in cows receiving 1 kg/d of supplemental fat compared with controls. Responses to lipolytic or insulin challenges were not affected by feeding oil. Supplemental fat did not affect the digestion of pasture fiber. The addition of energy in the form of partially hydrogenated fat to early lactation dairy cows fed primarily on pasture increased the yield of fat-corrected milk and milk fat content when it represented about 11% of the total metabolizable energy requirement of cows, without affecting milk protein content. The partial hydrogenation of a byproduct of the oil industry apparently prevented detrimental effects of fat supplementation on ruminal digestion.
(**Key words:** partially hydrogenated oil supplementation, grazing, milk fat composition, ruminal digestion)

Abbreviation key: C2:C3 = acetate:propionate, **EE** = ether extract, **FA** = fatty acids, **FCM** = 4% fat-corrected milk, **IVDMD** = in vitro DM digestibility, **IVOMD** = in vitro OM digestibility, **ME** = metabolizable energy, **MUN** = milk urea nitrogen, **PUN** = plasma urea nitrogen, **ST** = somatotrophin, **T0** = no supplemental fat, **T0.5** = 0.5 kg/d of supplemental fat, **T1** = 1.0 kg/d of supplemental fat, **WSC** = water soluble carbohydrates.

INTRODUCTION

The supplementation of diets with fat for dairy cows in early lactation can be used to maintain a high energy density of the diet, while avoiding ruminal acidosis and reductions in milk fat content that may result when excessive amounts of starch are fed to early lactation dairy cows. In confinement systems with nutritionally balanced diets rich in energy, milk production is often increased in response to lipid supplementation to high-yielding dairy cows in early lactation (Palmquist and Jenkins, 1980; Schingoethe and Casper, 1991; Chilliard, 1993). In grazing systems, the quality of the diet (pasture plus concentrate) is more variable, and milk production per cow may be lower because of the lower energy density of the forage. In such systems, the response to dietary fat may be different, because the specific nutrients that limit milk production will not be the same. The intake of energy is thought to be the most limiting nutrient for milk production in pasture-based

Received April 12, 2001.
Accepted October 23, 2001.
Corresponding author: G. A. Gagliostro; e-mail: ggagliostro@balcarce.inta.gov.ar.

systems (Muller and Fales, 1998). The benefit of supplemental fat in this nutritional context needs to be explored as most dairy systems in Argentina and in other temperate regions of the world integrate grazed pasture with the feeding of concentrates and by-products.

Experimental results about the effects of fat supplementation on dairy cow performances or BW losses in grazing conditions are scarce. King et al. (1990a) reported an increase in milk yield in pasture-fed dairy cows supplemented with long-chain fatty acids (**FA**). Feeding calcium salts of FA to early lactation dairy cows grazing an alfalfa-based pasture improved milk yield (+11%), milk fat yield (+14%), and milk protein production (+13%) without affecting parameters associated with body lipid mobilization (Gagliostro, 1998).

The metabolic effects of fat feeding in pasture-based diets are poorly described in the literature and may differ from those obtained in confinement systems due to the uncontrolled variability associated with the nutritive value of the pasture. In addition, the reduction of total DM and carbohydrate intake often observed after supplemental fat feeding may induce some energetic uncoupling due to an under-supply of ruminally available energy relative to the release of pasture-N in the rumen (Muller and Fales, 1998). In confined cows in early lactation, dietary lipids seem to be metabolized in order to support mammary gland requirements rather than to prevent the loss of BW (Chilliard, 1993). Several metabolic adaptations following fat feeding have also been reported in confined dairy cows. Increased β-adrenergic lipolytic responses (Gagliostro and Chilliard, 1991b) and enhanced losses of BW or BCS were observed in early lactation (Chilliard, 1993).

Fat feeding has been associated with lower milk protein content. Together with a dilution effect due to a higher milk yield, insulin resistance has also been proposed to explain the decrease in milk protein concentration (Palmquist, 1984; Wu and Huber, 1994). Development of insulin resistance was reported in cows that received lipids (Chilliard, 1993), although the response of plasma glucose and NEFA to insulin challenge was not altered by duodenal oil infusion in confined feeding systems (Gagliostro and Chilliard, 1991b) or by calcium salts of FA in grazing conditions (Gagliostro, 1998).

The effects of fat supplementation on regulatory plasma hormone concentrations are not consistent (Chilliard, 1993), and data regarding hormonal profiles after fat supplementation are not available for grazing dairy cows. The production responses or the metabolic adaptations after fat feeding may partly result from changes in circulating hormones (Chilliard, 1993; Staples et al., 1998).

Elevating the melting point above the ruminal temperature may be a suitable strategy for avoiding negative effects of fat on ruminal digestion (Chalupa et al., 1986) when applied to vegetable oil, making it acceptable for ruminant feeding.

The objective of this experiment was to determine whether the addition of energy in the form of fat to the concentrate of grazing dairy cows would increase milk production in early lactation without negative effects on milk composition or body condition parameters. Concentrations of plasma metabolites and hormones and responses to lipolytic or insulin challenges were also studied to better understand the performance of the cows. The effects of partially hydrogenated oil on ruminal environment and pasture digestion were also evaluated in grazing conditions.

MATERIALS AND METHODS

Cows and Diets

The experiment was conducted at the National Institute of Agricultural Technology in Balcarce (37°45′S, 58°18′W), Argentina, during spring 1997. Thirty-seven multiparous Holstein cows (570 ± 68 kg BW) were grouped based on number of lactations and milk production registered during the first 70 DIM for the previous lactation. They were randomly allocated to one of three dietary treatments: no supplemental fat (**T0**, 12 cows), 0.5 kg/d supplemental fat (**T0.5**, 12 cows), and 1 kg/d supplemental fat (**T1**, 13 cows) as partially hydrogenated oil. Fat was added to a basal concentrate composed of ground corn (91.6%), fish meal (8%), and calcium chloride (0.4%; Table 1).

The concentrates were offered in two equal feedings during milking times (0600 and 1600 h) from 19 ± 5 d (mean ± SD) before the expected calving date to 70 DIM for all treatments. After 70 DIM, all cows were fed the T0 concentrate for an additional period of 30 d to evaluate the residual effects of fat feeding on milk yield and composition. Intake of concentrate occurred daily and was individually recorded by weighing the amounts offered and refused.

Fat was a byproduct obtained when vegetable oil was purified for human nutrition. The oil residue was partially hydrogenated, leaving flakes composed of triglycerides of high melting point (58 to 60°C) that remained solid at ruminal temperature (39 to 40°C). The FA composition of the partially hydrogenated oil was $C_{14:0}$ (2.4%), $C_{14:1}$ plus iso $C_{15:0}$ (0.7%), $C_{15:0}$ (0.9%), $C_{15:1}$ (0.4%), $C_{16:0}$ (30.3%), $C_{16:1}$ (0.2%), $C_{17:0}$ (1.2%), $C_{17:1}$ (0.1%), $C_{18:0}$ (34.9%), $C_{18:1}$ (21.8%), $C_{18:2}$ (3.3%), $C_{18:3}$ (0.9%), and others (2.9%). A soluble source of Ca was added to the concentrate to compensate for any potential loss in Ca digestibility due to fat supplementation (Palmquist, 1984).

Table 1. Ingredient and nutrient composition of the experimental concentrates.

Item	Treatment		
	T0	T0.5	T1
Ingredient, kg DM/cow/d			
Ground corn	5	5	5
Fish meal	0.4	0.4	0.4
Calcium chloride	0.02	0.02	0.02
Fat[1]	0	0.5	1
Total offered	5.4	5.9	6.4
Nutrient			
DM, %	87.8	88.9	89.0
OM, % of DM	93.2	96.6	96.4
IVDMD,[2] %	85.0	84.6	84.3
CP, % of DM	18.8	12.9	12.5
NDF, % of DM	15.9	18.3	11.7
EE, % of DM[3]	4.5	13.3	18.3

[1]Partially hydrogenated oil. Melting point: 58 to 60°C. Fatty acid composition was 30.3% $C_{16:0}$, 34.9% $C_{18:0}$, 21.8% $C_{18:1}$, and 3.3% $C_{18:2}$.

[2]IVDMD = In vitro DM digestibility.

[3]EE = Ether extract.

All cows grazed together on mixed pastures that contained (DM basis) alfalfa (*Medicago sativa*, 46%), orchardgrass (*Dactylis glomerata* L., 49%) and dead material (4%). The area of the strip was regulated with a temporary electric fence to achieve a herbage allowance of about 30 kg DM/cow per d to allow a high pasture intake of cows (Minson, 1990). The cows were moved to a new grazing strip every day, and after grazing each strip was clipped of nongrazed forage to about 8 cm to allow a clean and uniform pasture regrowth.

Samples Collection and Analysis

After an adaptation period of 21 d (–14 to 7 DIM), milk production was daily and individually recorded. Milk samples were collected every 10 d at a.m. and p.m. milkings, composited according to the corresponding volume measured at each milking time and analyzed for fat, protein, lactose, and SNF by infrared spectrophotometry (Foss 605B Milko-Scan, Foss Electric, Hillerød, Demmark), and 4% FCM was calculated. Milk urea nitrogen (**MUN**; Wiener Laboratory, Rosario, Argentina) and cholesterol (Colestat, Wiener Laboratory, Rosario, Argentina) were determined with commercial enzymatic kits as described in Schroeder and Gagliostro (2000). Casein was determined as proposed in AOAC (1990).

Milk FA composition was analyzed in samples collected at 30 and 60 DIM. Milk fat was extracted at 100°C for 4 h with a Triton X-100 solution. When the sample was completely separated, it was removed from the oven, and the milk fat was transferred into a separatory funnel. The extracted fat was then purified with 30 ml of light petroleum ether and washed three times with 10 ml of isopropyl alcohol and sodium sulfate solution. The organic phase was then dried with sodium sulfate (2 spoonsful), the sample was filtered, and the solvent was removed with a rotary evaporator in a water bath at 40°C. Methyl esters were prepared by mixing the dried extract (45 mg of milk fat) with a 0.3 ml of sodium methanol solution (10 mg anhydrous sodium/1 ml of methanol). The mixture was incubated in a water bath at 67°C for 3 min and then cooled at room temperature (1 min). A spoonful of silica gel-$CaCl_2$ was added, and the mixture was vortexed for 30 s. The extracted fat was dissolved in 1.5 ml at CS_2 and centrifuged at 1800 rpm for 10 min. The upper layer (CS_2 with the methyl esters) was analyzed by GLC to determine the FA composition using a Varian 3700 gas chromatograph fitted with a stainless steel column packed with 20% BDS (Alltech Associates, Inc. Applied Science Labs. IL-USA) Chromosorb WAW (80–100 mesh) (Varian Instrument Div., 670 E. Arques, Sonnyvale, CA 94086 USA) and a flame-ionization detector. Chromatography conditions were as follows: the initial and final temperatures of the oven were 130 (2 min) and 200°C, respectively; the temperature gradient was 10°C /min; the carrier gas was N_2 (60 psi) at a rate flow of 25 ml/min; and a mixture of H_2 (40 psi) and air (60 psi) was used for the detector. The injection volume was 3 μl (IRAM 5650 Part II, modified by Centro de Investigaciones Tecnológicas de la Industria Láctea [ISO 9002], Buenos Aires, Argentina).

Total herbage mass was determined every 10 d by cutting 20 quadrats (0.1 m^2 per quadrat) of pasture samples to ground level. Each sample was dried at 60°C in a forced-air oven. The quality of the grazed herbage was estimated from samples obtained by hand-plucking

at random transects every 10 d. Samples of concentrates were collected every 30 d. Pasture and concentrates samples were dried (60°C in a forced-air oven), ground through a 1-mm screen (Wiley Mill, Philadelphia, PA), and analyzed for OM, NDF, and ADF (Goering and Van Soest, 1970), CP (AOAC, 1990), in vitro DM digestibility (**IVDMD**, Tilley and Terry, 1963), in vitro OM digestibility (**IVOMD**, Tilley and Terry, 1963), water-soluble carbohydrate (**WSC**; Morris, 1948), and ether extract (**EE**; AOAC, 1990).

Pasture DMI was estimated on eight cows by treatment from 55 to 70 DIM using Cr_2O_3 as an indigestible fecal marker. After each milking, cows were dosed twice daily with three gelatin capsules containing 2 g of Cr_2O_3 during a period of 14 d (12 g of Cr_2O_3/cow per d). Fecal grab samples were collected after milking on d 7 to 14. Total fecal DM production (kg/d) was estimated by dividing the total Cr dosed (g/d) by the Cr concentration in fecal DM (g/d) determined by absorption spectrophotometry. Fecal DM output due to concentrate was estimated as concentrate intake × (1–concentrate IVDMD). This quantity was subtracted from the total fecal DM production, and the remaining fecal DM was attributed to pasture. Pasture intake was calculated as the ratio between fecal DM yield due to pasture and pasture indigestibility (1–IVDMD). Total energy intake was calculated from DMI of forage and concentrates and their metabolizable energy (**ME**) content estimated according to NRC (2001). Lipid ME content (5.18 Mcal/kg DM) was calculated assuming that FA digestion in the total tract was 56.5% for total C_{16} and 63.6% for total C_{18} (Pantoja et al., 1995).

Cows were weighed on 2 consecutive d after the a.m. milking at the start (4 and 5 DIM), in the middle (30 and 31 DIM), and at the end (69 and 70 DIM) of the period of fat supplementation. An additional record of BW was taken at the end of the residual period (99 and 100 DIM). On the same days, BCS was also recorded by two independent observers using the five-point scale (1 = thin to 5 = fat). The mean value of the two records was used to calculate changes in BW gain or BCS.

Blood samples were collected from the jugular vein immediately after the a.m. milking at 15, 30, 45, and 60 DIM. On the days of sampling, cows received the concentrates immediately after bleeding. On d 34 of lactation, blood samples were taken before and 15 min after a β-adrenergic agent (Isoproterenol) challenge (4 nmol/kg BW, Proterenal, Phoenix Laboratory, Buenos Aires, Argentina [Gagliostro and Chilliard, 1991b]). On d 37 of lactation, blood samples were taken before and 15, 30, and 60 min after a challenge with bovine insulin intravenously (0.12 U/kg BW, Betasint, Beta Laboratory, Buenos Aires, Argentina [Gagliostro and Chilliard, 1991b]). For every sample, blood was collected in heparinized tubes (Abbot Laboratory, Argentina, 5 U/ml) and immediately placed on ice. Plasma was obtained (2000 × *g* at 4°C for 10 min) and stored frozen (–24°C) until analysis. Commercial enzymatic kits were used for plasma urea nitrogen (**PUN**; Wiener Laboratory, Rosario, Argentina [Physiol. Chem. 329, 2141, 1962]), NEFA (Wako Pure, Chemical Industries USA, Inc., Dallas, TX), glucose (Wiener Laboratory, Rosario, Argentina), triglyceride (Wiener Laboratory, Rosario, Argentina), and total cholesterol (Wiener Laboratory, Rosario, Argentina) as described in Schroeder and Gagliostro (2000). In samples taken at 30 and 60 DIM, plasma IGF-I was measured by radioimmunoassay with previous acid-ethanol extraction (Lacau-Mengido et al., 2000). Insulin-like growth factor-I antibody (UB2-495; Hormone Distribution Program, National Institute of Diabetes and Digestive and Kidney Diseases, Rockville, MD) was used. The intraassay coefficient of variance was 7%, and assay sensitivity was 60 pg/tube. Somatotrophin (**ST**) and insulin were measured in the same samples. Concentration of ST was determined by radioimunoassay using an antiovine antibody (Díaz-Torga et al., 2001). The intraassay coefficient of variance was 7.2%, and minimum detectable concentration was 0.8 ng. Insulin was measured by radioimmunoassay using anti-bovine insulin antibody (Sigma, St. Louis, MO) and standard human insulin provided by Laboratories Beta (Buenos Aires, Argentina [Díaz-Torga et al., 2001]). Minimum detectable concentration was 0.05 ng.

Rumen Environment and in Situ Pasture NDF Degradability

The effects of fat supplementation on ruminal environment and parameters of pasture NDF degradation were evaluated using six lactating Holstein cows fitted with ruminal cannulas in a replicated 3 × 3 Latin square design with 15-d periods. Three cows in early lactation and another three cows in midlactation were used. These cows received the same treatments and were in a single herd under strip grazing conditions together with nonfistulated cows. During the 15th d of each experimental period, samples of ruminal content were taken from the dorsal, ventral, and caudal areas at 0 (6:00 a.m.), 4, 8, 12, 16, and 20 h after the first sample. Ruminal fluid was obtained by straining through four layers of cheesecloth, and pH was measured immediately (Orion portable pH meter 250A, Orion Research Inc., Boston, MA). A sample (100 ml) was acidified with 1 ml of 1 *N* H_2SO_4 and frozen (–24°C). Samples were later thawed and centrifuged at 15,000 × *g* for 15 min (4°C), and the supernatants were analyzed for concentrations of NH_3-N (Autoanalyser Technicom, model 2)

and VFA. The VFA were determined in a gas cromatograph (Gow-Mac 69-750P) using N_2 as a carrier gas at a flow rate of 24 ml/min and a glass column packed with 80/120 Carbopack B-DA/4% Carbowax 20M (Supelco, Inc., Bellefonte, PA). Temperatures for the oven, injector port, and detector were 175, 200, and 200°C, respectively.

Pasture samples were obtained by hand-plucking and were cut to a final length of 1 cm. The wet material was immediately placed (5 g of DM/bag) in Dacron bags (15.5 × 7.5 cm, 52 μm pore size, Ankom, Fairport, NY). The bags were incubated in the ventral sac of the rumen by duplicate for 0, 4, 8, 12, 16, 20, 26, 32, 40, and 48 h. After incubation, the bags were rinsed in a pipette washer for 1 h and then hand-washed with cold tap water. Bags were squeezed until the water was clear and then oven dried at 60°C until constant weight. The residues from each bag were weighed, ground through a 1-mm screen, pooled within cow for each time of incubation, and analyzed for NDF content. Kinetic parameters of ruminal NDF degradation were estimated with the equation proposed by Ørskov and McDonald (1979): $D = A + B\ (1\text{-}e^{-kdt})$, where D = disappearance at time (t), A = soluble fraction (%, wash value at 0 h), B = insoluble potentially digestible fraction (%), kd = fractional rate of degradation (%/h), and t = time of incubation. Total potentially degradable fraction of NDF was estimated as A + B. All the parameters of the model were estimated with a nonlinear model using the NLIN procedure of SAS (1996) and the Marquardt iterative method. The effective degradation of NDF was calculated with the following equation: effective degradation = A + B (kd/(kd + kp)), where kp = rate of passage (assumed to be 0.05 and 0.07/h; Ørskov and McDonald, 1979).

Statistical Analysis

Milk production and composition, changes in BW and BCS, and plasma metabolite and hormone concentration were evaluated by the PROC MIXED of SAS (1996) for repeated measurements using the following model:

$$Y_{ijk} = \mu + T_i + A_{(i)j} + D_k + (T \times D)_{ik} + \varepsilon_{ijk}$$

where

Y_{ijk} = the dependent variable,
μ = overall mean,
T_i = treatment effects,
A_{ij} = random effects of animal within treatments,
D_k = effects of sampling date or time,
$(T \times D)_{ik}$ = interaction effects of treatment and sampling date or time, and
ε_{ijk} = the residual error associated with the ijk observation.

Milk yield was also analyzed using the same model but introducing the average milk production obtained over the first 70 DIM of the previous lactation as a covariate.

Data from DMI and milk FA composition were analyzed with the GLM procedure of the SAS (1996) program using the following model:

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

where

Y_{ij} = the dependent variable,
μ = overall mean,
T_i = treatment effects, and
ε_{ij} = residual error.

The rumen parameters were analyzed in a replicated 3 × 3 Latin square, one square with three cows in early lactation and the other with three cows in midlactation. Data were analyzed using the GLM procedure of the SAS (1996) program using the following model:

$$Y_{ijkt} = \mu + S_i + C_{(i)j} + P_{(i)k} + T_t + T \times S_{it} + \varepsilon_{ijkt},$$

where

Y_{ijkt} = the dependent variable,
μ = overall mean,
S_i = square (early or mid-lactation) effects,
$C_{(i)j}$ = the effects of cows within the square,
$P_{(i)k}$ = the effects of the period within the square,
T_t = treatment effects,
$T \times S_{it}$ = the interaction effects of treatments by square, and
ε_{ijkt} = residual error.

Mean comparisons were carried out using the Tukey-Kramer test, and differences were considered significant with $P < 0.10$ unless otherwise stated.

RESULTS

Pasture Characteristics and DMI

The average value (± standard deviation) of herbage mass in the pregrazing strips was 1968 ± 821 kg DM/ha, and the average herbage allowance obtained was 30 ± 8 kg DM/cow per day during the trial. Values for the chemical composition of the forage apparently consumed by cows are shown in Table 2.

Table 2. Nutrient composition of spring pastures during the experiment.[1]

		% of DM							
	% DM	OM	IVDMD[2]	IVOMD[2]	NDF	ADF	CP	WSC[2]	EE[2]
Average	24.4	90.5	73.2	72.5	38.3	21.3	23.4	9.5	3.7
SD	1.6	1.0	3.9	5.0	5.6	4.5	1.3	3.4	1.1
Minimum	23.0	88.5	65.2	64.4	30.5	17.0	22.4	5.6	2.3
Maximum	26.6	91.5	75.2	77.0	45.0	26.8	25.7	14.1	5.0

[1]Samples were obtained by hand-plucking from August to December.

[2]IVDMD = In vitro DM digestibility, IVOMD = in vitro OM digestibility, WSC = water soluble carbohydrates, EE = ether extract.

Milk Production and Composition

The yield of FCM over the experimental period is presented in Figure 1. An interaction between treatment and week of lactation was not detected ($P < 0.62$). The peak of FCM yield was observed between the 4th and 5th wk of lactation (Figure 1). At that moment, FCM resulted higher in T0.5 (+8.2%) and in T1 (+14.8%) compared with control (T0) cows. Average values for milk production, milk composition, and yield of milk components over the fat supplementation period (first 70 DIM) are presented in Table 3.

No interactions between treatment and week of lactation were detected for any parameter measured (Table 3). During the first 70 DIM, the cows produced similar amounts of milk. Milk fat content, FCM yield, and milk fat production were higher in T1 compared with control, but milk protein yields were not affected (Table 3). With data for milk yield recorded during the first 70 DIM of the previous lactation as covariate ($P < 0.01$), the average FCM was higher ($P < 0.05$) in T1 (26.3 kg/d) compared with T0 (23.5 kg/d) with no differences between these treatments and T0.5 (24.6 kg/d). Percentages of milk protein, casein, MUN, and total cholesterol and daily yields of protein were not affected (Table 3).

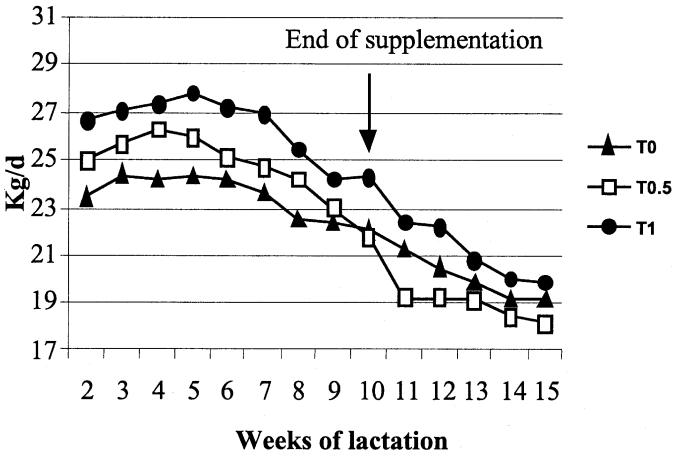

Figure 1. Yield of FCM in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil during the first 70 d of lactation. Treatment by wk of lactation interaction = $P < 0.62$, wk = $P < 0.01$ and treatment = $P < 0.06$.

The amounts of concentrate and pasture consumed by cows are shown in Table 4. As fat was added to the basal concentrate (without replacing corn; Table 1) and differences in concentrate refusals were not observed among T0 (0.22 kg DM/cow per d), T0.5 (0.38 kg DM/cows per d), and T1 (0.40 kg DM/cows per d; $P < 0.71$), the cows from T1 showed the highest concentrate DMI (Table 4). Compared with T0, cows from T1 showed a lower pasture DMI, but no differences in total DM or total estimated energy intake were detected (Table 4).

Average values for milk production and composition and yield of milk components over the residual period (70 to 100 DIM) are presented in Table 5. During the residual period when all cows received the T0 concentrate, the yield of FCM was higher ($P = 0.06$) in T1 compared with T0.5 without differences between these two treatments and T0 (Table 5). There was an interaction ($P < 0.05$) between treatment and week for milk fat secretion (Table 5). Cows from T1 produced more ($P < 0.05$) milk fat during the first 4 wk of the residual period compared with T0.5, but the differences disappeared thereafter. No residual effects of fat supplementation were detected for any other parameter measured (Table 5). Milk FA composition is shown in Table 6.

An increase in milk fat percentages of $C_{16:0}$ and $C_{18:0}$ acids with a compensatory decline of $C_{18:2}$, $C_{18:3}$, and short and medium-chain FA was detected ($P < 0.05$) in the T1 cows (Table 6).

The individual FA yield is shown in Table 7. The yields of $C_{16:0}$ and $C_{18:0}$ acids were higher in T1 compared with the other treatments. Yield of $C_{18:1}$ acid was increased in T1 compared with T0 treatment (Table 7).

Changes in BCS and BW

The changes in BCS and BW during and after the period of fat supplementation are shown in Figure 2

Table 3. Average values of milk production and composition in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil during the first 70 d of lactation.

	Treatment[1]				$P <$[2]		
Item	T0	T0.5	T1	SD	Treat	Wk	Treat × Wk
Milk, kg/d	25.6	26.9	27.2	3.8	0.39	0.01	0.41
FCM, kg/d	23.4[b]	24.6[ab]	26.3[a]	3.7	0.06	0.01	0.62
Milk fat							
kg/d	0.87[b]	0.93[ab]	1.03[a]	0.16	0.01	0.01	0.63
%	3.44[b]	3.51[b]	3.78[a]	0.60	0.03	0.01	0.45
Milk protein							
kg/d	0.83	0.87	0.86	0.12	0.55	0.01	0.63
%	3.24	3.32	3.18	0.34	0.33	0.01	0.32
Casein, % of total N	70.4	68.2	70.3	5.5	0.94	. . .	. . .
Lactose, %	5.13	5.02	5.15	0.23	0.19	0.01	0.61
Total solids, %	12.5	12.5	12.6	0.8	0.81	0.01	0.52
Solids non-fat, %	8.95	8.90	8.89	0.40	0.89	0.01	0.18
MUN,[3] mg/dl	22.1	21.6	19.8	12.91	0.61	0.01	0.28
Cholesterol, mg/dl	47.0	56.7	42.6	26.3	0.16	0.02	0.63

[a,b]Means within a row that have different superscripts are significantly different ($P < 0.10$).

[1]n = 12, 12, and 13 for T0, T0.5, and T1, respectively.

[2]Effects of treatment (Treat), week of lactation (Wk) and interaction treatment by week (Treat × Wk).

[3]MUN = Milk urea nitrogen.

and Table 8, respectively. No significant differences were observed in the losses of BCS and BW gain during the period of fat supplementation. After the end of fat feeding at 11th wk of lactation, the cows began to gain BCS and BW in all treatments (Figure 2).

Plasma Metabolites and Hormones

Plasma metabolite and hormone concentrations are presented in Table 9. Average plasma levels of glucose, NEFA, and PUN were not affected by fat feeding. Concentrations of plasma triglyceride were lower and total cholesterol was higher in T1 compared with T0 ($P < 0.05$; Table 9). Plasma levels of insulin were lower ($P < 0.01$) in T1 than in the other treatments, whereas plasma ST and IGF-I concentrations did not change (Table 9). Plasma ST and IGF-I concentrations were higher on d 60 (2.7 and 426 ng/ml, respectively) than on d 30 (ST = 2.4 and IGF-I = 332 ng/ml) of lactation, whereas plasma insulin decreased with the advance of lactation (1.39 vs. 0.38 ng/ml on d 30 and 60, respectively).

Changes in plasma metabolite concentration after isoproterenol challenge are shown in Table 10. There was no interaction between treatment and sampling time for any of the metabolites measured (Table 10). The basal plasma NEFA and glucose concentrations and the responses to challenges by isoproterenol were

Table 4. Concentrate, pasture, and total DM and energy intake in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil.

	Treatment[1]				
Intake	T0	T0.5	T1	SD	$P <$
Concentrate intake, kg DM/d	5.2[b]	5.5[ab]	6.0[a]	0.4	0.01
Pasture intake, kg DM/d	17.8[a]	14.3[ab]	13.6[b]	3.3	0.05
Total DMI, kg/d	23.0	19.8	19.6	3.1	0.11
Total NE_L intake, Mcal/d[2]	36.7	33.9	34.5	4.2	0.42
Total NE_L requirement, Mcal/d[2]	29.6	30.9	31.2	2.8	0.55
Energy balance, Mcal/d[2]	7.06	3.05	3.27	3.54	0.08
DMI/FCM	1.13[a]	0.88[b]	0.84[b]	0.13	0.01
FCM/NE_L intake	0.56[b]	0.68[ab]	0.69[a]	0.08	0.04

[a,b]Means within a row that have different superscripts are significantly different ($P < 0.10$).

[1]n = 8, 8, and 7 for T0, T0.5, and T1, respectively.

[2]Estimated according to NRC (2001) using data obtained during the period of pasture intake measurements (55 to 70 DIM). Lipid ME content (5.18 Mcal/kg DM) was calculated assuming that FA digestion in the total tract were 56.5% for total C16 and 63.6% for total C18 (Pantoja et al., 1995).

Table 5. Average values of milk production and composition in grazing dairy cows after supplementation with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil (70 to 100 DIM).

	Treatment[1]				$P <$[2]		
Item	T0	T0.5	T1	SD	Treat	Wk	Treat × Wk
Milk, kg/d	23.2	22.3	23.7	3.0	0.39	0.01	0.15
FCM, kg/d	21.1[ab]	20.6[b]	22.9[a]	2.6	0.06	0.01	0.11
Milk fat							
kg/d	0.73	0.65	0.78	0.15	0.10	0.01	0.04
%	3.07	3.01	3.27	0.54	0.35	0.79	0.42
Milk protein							
kg/d	0.72	0.71	0.72	0.07	0.96	0.01	0.16
%	3.08	3.18	3.03	0.22	0.24	0.02	0.64
Lactose, %	5.02	4.89	4.91	0.31	0.47	0.52	0.38
Total solids, %	11.9	11.8	12.0	0.6	0.85	0.13	0.25
Solids non-fat, %	8.72	8.74	8.60	0.38	0.59	0.87	0.17
MUN,[3] mg/dl	8.61	9.89	13.20	6.1	0.13	0.01	0.53
Cholesterol, mg/dl	41.6	48.7	42.1	14.7	0.51	. . .	. . .

[a,b]Means within a row that have different superscripts are significantly different ($P < 0.10$).

[1]n = 12, 12, and 13 for T0, T0.5, and T1, respectively.

[2]Effects of treatment (Treat), week of lactation (Wk) and interaction treatment by week (Treat × Wk).

[3]MUN = Milk urea nitrogen.

not affected by fat feeding (Table 10). When the increase in NEFA and glucose concentrations 15 min after the isoproterenol injection was adjusted by subtracting the basal (preinjection) concentrations, the increase for both metabolites was not significantly different between treatments. The in vivo hypoglycemic action of insulin is shown in Figure 3.

After insulin challenge, the decrease in plasma glucose concentration was not affected by supplemental fat. Interactions between treatment and sampling time were not detected (Figure 3). The in vivo antilipolytic action of insulin is shown in Figure 4.

After insulin injection, plasma concentration of NEFA decreased for all treatments, but a less pro-

Table 6. Milk fatty acid composition in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation.

	Treatment[1]				
	T0	T0.5	T1	SD	$P <$
Fatty acid, %					
C4:0	2.6	2.3	2.3	0.4	0.16
C6:0	1.9[a]	1.7[ab]	1.6[b]	0.3	0.02
C8:0	1.2[a]	1.0[ab]	0.8[b]	0.2	0.01
C10:0	2.3[a]	2.0[ab]	1.7[b]	0.5	0.02
C10:1	0.2[a]	0.2[ab]	0.1[b]	0.1	0.02
C12:0	2.6[a]	2.3[ab]	1.9[b]	0.6	0.02
C12:1	0.2	0.2	0.1	0.1	0.10
C14:0	8.8[a]	8.0[ab]	7.3[b]	1.4	0.05
C14 iso 15	1.9[a]	1.7[ab]	1.5[b]	0.2	0.01
C15:0	1.4	1.4	1.3	0.2	0.42
C15 iso 16	0.6	0.6	0.5	0.2	0.43
C16:0	22.3[b]	23.6[ab]	25.5[a]	2.2	0.01
C16:1	2.8	2.9	2.4	1.0	0.50
C17:0	1.3	1.4	1.2	0.4	0.67
C17:1	1.2	1.0	0.8	0.5	0.17
C18:0	11.9[b]	12.5[ab]	14.0[a]	1.9	0.02
C18:1	28.9	30.7	30.6	3.0	0.30
C18:2	4.6[a]	4.2[ab]	3.8[b]	0.5	0.01
C18:3	2.5[a]	2.0[b]	1.7[b]	0.5	0.01
Others	0.94	0.63	0.76	0.58	0.44

[a,b]Means within a row that have different superscripts are significantly different ($P < 0.10$).

[1]n = 12, 12, and 12 for T0, T0.5, and T1, respectively.

Table 7. Yield of individual milk fatty acids (FA) in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation.

	Treatment[1]				
FA (g/d)	T0	T0.5	T1	SD	$P <$
C4:0	22.5	21.6	23.9	5.1	0.56
C6:0	16.5	15.9	16.3	3.5	0.89
C8:0	10.0	9.3	8.7	2.6	0.50
C10:0	20.2	18.5	17.6	5.7	0.54
C10:1	2.0	1.7	1.5	0.8	0.21
C12:0	22.5	21.3	19.6	6.2	0.52
C12:1	1.3	1.4	1.0	0.6	0.33
C14:0	76.9	75.6	76.4	18.7	0.99
C14 iso 15	15.9	16.3	15.4	3.1	0.78
C15:0	11.7	13.1	13.3	2.4	0.23
C16:0	194.2[b]	224.1[b]	263.7[a]	41.5	0.01
C16:1	23.7	27.6	23.9	10.0	0.58
C17:0	11.0	13.1	12.4	4.1	0.45
C18:0	102.3[b]	119.9[b]	144.2[a]	25.4	0.01
C18:1	250.5[b]	293.9[ab]	315.0[a]	53.0	0.02
C18:2	39.4	40.3	39.7	7.8	0.96
C18:3 group	21.6	19.1	17.8	5.4	0.23

[a,b]Means within a row that have different superscripts are significantly different ($P < 0.10$).

[1]n = 12, 12, and 12 for T0, T0.5, and T1, respectively.

Table 8. Changes in BW gain (BWG) in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation.

	Treatment[1]				
BWG, kg/d	T0	T0.5	T1	SD	$P <$
4 to 30 DIM	–0.14	–0.23	–0.21	1.03	0.98
30 to 70 DIM	–0.34	0.32	–0.26	0.96	0.34
70 to 100 DIM	0.85	0.72	0.98	0.70	0.85

[1]n = 12, 12, and 13 for T0, T0.5, and T1, respectively.

Table 9. Plasma metabolites and hormones in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5) and 1 kg/d (T1) of partially hydrogenated oil in early lactation.[1]

	Treatment[1]				$P <$[2]		
	T0	T0.5	T1	SD	Treat	Wk	Treat × Wk
Glucose, mg/dl	59.5	60.3	58.6	7.2	0.46	0.01	0.05
NEFA, μeq/L	562.2	581.5	589.7	220.3	0.94	0.01	0.36
PUN,[3] mg/dl	11.3	11.4	11.5	2.8	0.95	0.01	0.55
Triglycerides, mg/dl	318.5[a]	292.2[ab]	271.2[b]	69.0	0.05	0.01	0.87
Cholesterol, mg/dl	185.0[b]	217.7[ab]	235.8[a]	63.0	0.02	0.01	0.64
ST,[3] ng/ml	2.4	2.6	2.7	0.8	0.45	0.07	0.41
IGF-I, ng/ml	415.5	379.7	344.3	154.2	0.43	0.01	0.64
Insulin, ng/ml	0.96[a]	1.06[a]	0.50[b]	0.87	0.02	0.01	0.39

[a,b]Means within a row that have different superscripts are significantly different ($P < 0.10$).

[1]n = 11, 11, and 12 for T0, T0.5, and T1, respectively.

[2]Effects of treatment (Treat), week of lactation (Wk) and interaction treatment by week (Treat × Wk).

[3]PUN = Plasma urea nitrogen; ST = somatotrophin.

Table 10. Plasma NEFA and glucose concentrations before and 15 min after an intrajugular isoproterenol injection (4 nmol/kg BW) in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation.[1]

	Treatment				$P <$[2]		
	T0	T0.5	T1	SD	Treat	Time	Treat × Time
NEFA (μeq/L)					0.84	0.01	0.21
Basal (before injection)	594.6	645.4	602.6	198.8			
15 min after injection	969.4	885.4	850.2	282.1			
Glucose (mg/dl)					0.48	0.01	0.45
Basal (before injection)	56.1	55.2	60.9	7.1			
15 min after injection	65.2	68.7	68.0	11.5			

[1]Samples were taken on d 34 of lactation (n = 11, 11, and 12 for T0, T0.5, and T1, respectively).
[2]Effects of treatment (Treat), minutes after injection (Time) and Treat × Min interaction.

nounced decrease in concentration was not observed in any treatment. Interactions between treatment and sampling time were not detected (Figure 4).

Ruminal Environment and Forage NDF Disappearance

Parameters of rumen environment measured in the fistulated cows are presented in Table 11. Treatment × square (stage of lactation) interaction was not observed. The overall means for total or individual VFA concentrations, for the acetate:propionate (**C2:C3**) ratio, for the concentrations of NH_3-N or the pH values were not affected by fat supplementation (Table 11).

Parameters of in situ disappearance of forage NDF are shown in Table 12. The addition of partially hydrogenated oil to the diet did not affect the degradable NDF fraction, the rate of NDF degradation, or the effective NDF degradability of the pasture (Table 12). The potentially digestible fraction of forage NDF was 77.1% in T0, 73.9% in T0.5, and 76.5% in T1 ($P < 0.72$).

DISCUSSION

Diet Characteristics and Feed Intake

In this experiment, a daily strip-grazing system was used, and cows were allocated to areas of sufficient size to provide about 30 kg/d DM of pasture per cow with an herbage biomass averaging 2000 kg DM/ha. Minson (1990) suggested that these conditions avoid a possible reduction in voluntary DMI of pasture. In control cows that showed the highest pasture DMI (Table 4), NDF intake was close to the value (12.5 g kg^{-1} BW d^{-1}) proposed by Mertens (1994) to obtain maximum DMI. The NDF concentration of forage (Table 2) was below the values (40 to 50%) considered as adequate for well-managed pastures (Minson, 1990; Muller and Fales, 1998) and close to the value of 34 to 36%, which would not affect voluntary DMI due to rumen fill (Mertens, 1994). Average pasture CP content (Table 2) was in the range of 15 to 25% proposed by Minson (1990) to obtain high values of forage DM digestibility and contributed to maintain sufficient ruminal NH_3-N concentrations

Table 11. Parameters of rumen environment in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil.

	Treatment				
	T0	T0.5	T1	SEM	$P <$
VFA, mmol/L					
Total	88.3	85.8	79.3	5.92	0.56
Acetate (C2)	56.4	53.6	49.7	4.04	0.52
Propionate (C3)	16.8	17.2	16.2	1.07	0.80
Isobutyrate	1.40	1.31	1.18	0.1	0.34
Butyrate	10.5	10.3	9.30	0.76	0.51
$2CH_3$ Butyrate	0.95	0.94	0.82	0.06	0.27
Isovalerate	0.95	0.96	0.80	0.07	0.26
Valerate	1.29	1.39	1.32	0.16	0.90
C2:C3 ratio	3.32	3.19	3.12	0.12	0.51
NH_3-N, mg/dl	17.8	18.1	14.5	1.5	0.25
pH	5.73	5.74	5.74	0.05	0.98

(Table 11) to support optimal microbial growth (Satter and Slyter, 1974). All these data suggested that both quality and quantity of pasture was adequate throughout the experiment.

Concentrate refusals were not increased by the addition of fat. Reductions in concentrate intake when fat is added have been attributed to a lower rate of intake and to reduced palatability (Grummer et al., 1990). This fact could represent a serious problem in grazing cows that receive the concentrate for a limited time during each milking. In T1, concentrate intake was not reduced even when fat was added up to 18.5% of concentrate DM value that resulted close to the range of 22 to 30% proposed by King et al. (1990b) in which concentrate intake may be depressed.

Cows in treatment T1 consumed less forage than control cows (Table 4). This finding could not be explained by negative effects of supplemental fat on ruminal digestion. In fact, the parameters of rumen environment (Table 11) or forage NDF disappearance (Table 12) were not affected by fat addition. Because pasture DMI was decreased in T1 but energy intake from the concentrate was increased by adding fat, estimated total energy intake was similar among treatments (Table 4). These results suggest that energy-homeostatic mechanisms were probably involved in regulating DMI as observed in dairy cows in midlactation when rapeseed oil was infused into the duodenum (Gagliostro and Chilliard, 1991a). These results agree with other studies in which a metabolic regulation of DMI was apparent after feeding protected fat (Choi and Palmquist, 1996; Rodriguez et al., 1997).

Milk Production and Composition

There was a numerical increase in milk yield at the peak of lactation (+14.2%) and in the average milk production over the first 70 DIM (+6%) in T1 cows compared with control cows, but these differences were not significant (Table 3). The lack of significant effects on milk production may be attributed to the moderate number of replications and the high variation observed in milk yield (Table 3). The failure of supplemental fat to increase total energy intake of cows (Table 4) could also be involved, as milk yield is related to energy intake. When results were compared as FCM, a significant difference between T1 and T0 was detected (Figure 1 and Table 3), and the FCM response to added fat became apparent from the first weeks of lactation (Figure 1). It is not clear if feeding fat before calving might induce a postcalving lactation response. In lactating dairy cows, a 2- or 3-wk lag time was observed before milk yield responds to dietary fat independently of the energy intake of cows (Chilliard, 1993). Komaragiri et al. (1998) observed a numerical improvement in the yield of FCM (+2.9 kg/d) when cows were fed Ca salts of long-chain FA from 3 wk prior to expected calving date up to 12 wk of lactation without changes in either total DMI or body fat mobilization. In our experiment, it seems that the higher FCM yield was explained mainly by an increase in milk fat production (18%) rather than a higher milk yield (6%; Table 3). Incorporation of exogenous FA into milk fat increased the efficiency of milk fat secretion (Pantoja et al., 1995). To the extent that the covariate analysis performed removed any putative differences in milk production potential among the treatment groups at the start of lactation, it can be concluded that fat supplementation from about 19 d before the expected calving date to 70 DIM significantly increased FCM production in T1. Data obtained during the period of pasture intake measurements (55 to 70 DIM) were used to calculate the efficiency of FCM production using the equations proposed in NRC (2000). Cows receiving fat produced more FCM per kilogram of total DMI or per megacalorie of NE_L intake (Table 4). The improvement in the efficiency of energy utilization for lactation when fat is added to the diet has been attributed to a lower energy loss as methane, a greater efficiency arising from the direct use of long-chain FA for milk fat secretion and a higher efficiency of ATP

Table 12. Parameters of forage NDF degradation in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil.

	Treatment				
	T0	T0.5	T1	SEM	$P <$
NDF, %					
Soluble fraction	0.25	0.32	0.99	0.37	0.36
Degradable fraction	76.9	73.6	75.5	1.59	0.41
Rate of digestion, %/h	5.46	6.03	5.91	0.59	0.78
Effective NDF Degradability					
kp[1] = 5%/h	38.7	38.5	39.66	1.02	0.72
kp = 7%/h	32.5	32.6	33.6	1.0	0.72

[1]kp = Rates of passage assumed.

generation from long-chain FA rather than acetate (Chilliard, 1993; Garnsworthy, 1997). In other grazing experiments, FCM production was increased when supplemental fat was fed to dairy cows in early lactation (Gagliostro, 1998; Salado, 2000). It has been proposed that maximum efficiency of milk production occurs when about 12 to 16% of total ME requirements are supplied as dietary fat (Brumby et al., 1978). In the present experiment, ME provided by supplemental fat was about 11% in T1, a figure close to the range proposed as the most efficient for milk production. In T0.5, ME supplied by supplemented fat (about 5.8%) was considerably lower than the optimum suggested, and this could probably explain the lack of response on milk production (Table 3).

It has also been suggested (Palmquist, 1984; Schingoethe and Casper, 1991) that supplemental fat in early lactation may improve the persistency of milk and milk fat production through a carryover effect that may occur after the end of fat feeding. According to these observations, when fat supply was stopped at 70 DIM, FCM and milk fat yields remained higher in T1 than in T0.5 during the following 30 d of measurements (Figure 1 and Table 5). In our experiment, the effect of fat on persistency could be expected, because supplemented cows from T1 peaked at higher values of FCM (Figure 1), and the withdrawal of fat occurred when cows were already in positive energy balance (10 wk of lactation, Table 4). These carryover effects indicate that metabolic responses of cows to fat supplementation may persist beyond the supplemental period, suggesting the need to evaluate responses to fat feeding in long-term experiments (Palmquist and Jenkins, 1980; Schingoethe and Casper, 1991).

In the present study, total milk fat output was higher in T1 than in T0 (Table 3), with a higher yield of $C_{16:0}$, $C_{18:0}$, and $C_{18:1}$ FA and no changes in the secretion of short- and medium-chain FA (Table 7). Total milk fat production depends on the balance between the increase in exogenous FA transfer to the mammary gland and the decrease in de novo synthesis. A reduction of FA synthesis within the mammary gland is often observed when supplemental fat is added to the diet of lactating dairy cows (Garnsworthy, 1997). The reduction may be mediated at the ruminal level through a lower rumen acetate and butyrate production or through the inhibitory effect of long-chain FA on mammary lipogenic enzymes (Garnsworthy, 1997). In our study, no changes in the acetate and butyrate proportions or in the C2:C3 ratio were observed in the fistulated cows after fat feeding (Table 11). In spite of the lower pasture DMI observed in cows from T1 (Table 4), a limiting amount of acetate and butyrate production can apparently be excluded since a reduced mammary secretion of short- and medium-chain FA was not observed. The saturated fat supplement used in our experiment could also contribute to maintain the secretion of short- and medium-chain FA, because the inhibition of de novo mammary synthesis is more sensitive to unsaturated FA supply (Palmquist, 1984; Garnsworthy, 1997).

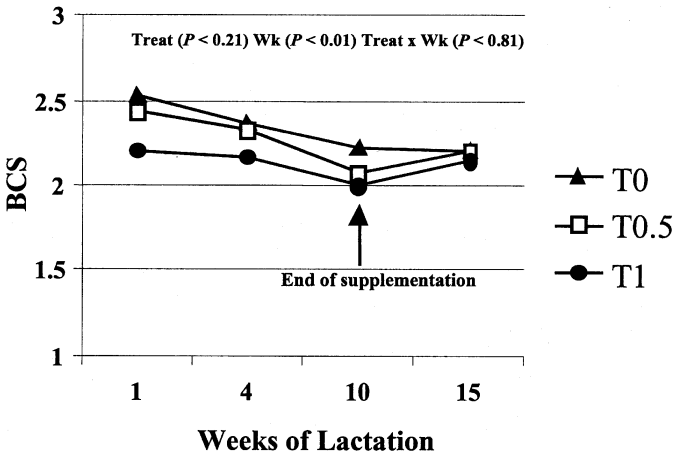

Figure 2. Changes in BCS in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation.

In our experiment, fat supplementation did not affect milk protein content or yield (Table 3). Reductions in milk protein content with fat feeding have been attributed to decreased glucose availability, development of insulin resistance, increased efficiency of milk production (decreased blood flow), or reduced plasma ST (Wu and Huber, 1994). Neither basal glucose concentration (Table 9) nor the in vivo hypoglycemic action of insulin (Figure 3) were affected by fat supplementation; in ad-

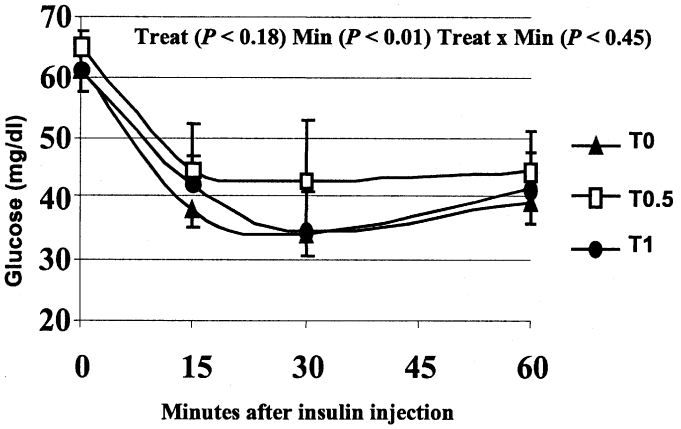

Figure 3. Concentrations of plasma glucose before and after an intrajugular insulin injection in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation. Samples were taken on d 37 of lactation.

dition, plasma ST concentration was not affected (Table 9). These metabolic results are consistent with the absence of negative effects of supplemental fat on milk protein (Table 3). The lower pasture DMI observed in T1 probably decreased microbial protein synthesis in the rumen. If so, the inclusion of fish meal as a source of RUP seemed to be effective in preventing a possible decrease in AA availability to the mammary gland. Other results obtained in grazing conditions (King et al., 1990a; Gagliostro 1998; Salado, 2000) showed no decrease in milk protein content when saturated fat was added to the diet.

Increments in all plasma lipids after fat supplementation have often been reported, but possible exceptions are triglycerides, owing to their rapid turnover rate (Christie, 1981). This fact, and the higher milk fat secretion observed in T1, may explain the lower plasma triglyceride concentration observed in this treatment (Table 9). In spite of the increase in plasma cholesterol levels in T1 (Table 9), milk cholesterol content was not affected, suggesting reduced mammary uptake of this metabolite (Christie, 1981).

Changes in BCS, BW, and Lipolytic Responses

Dietary fat does not seem to decrease the loss of BW, BCS, or lipid mobilization in early lactation cows (Palmquist, 1984; Gagliostro and Chilliard, 1991b; Chilliard, 1993; Komaragiri et al., 1998). Results obtained in our experiment are in accordance with this observation because fat supplementation did not change variations in BCS (Figure 2) or in BW (Table 8). Total energy intake was not increased (Table 4), but a higher response in FCM yield was observed in T1 cows (Table 3). As the increase in FCM yield was mainly explained by a higher milk fat content rather than milk yield (Table 3) and as basal NEFA concentrations remained unchanged (Table 9), energy balance was probably not decreased even when pasture DMI was reduced in cows from T1 (Table 4).

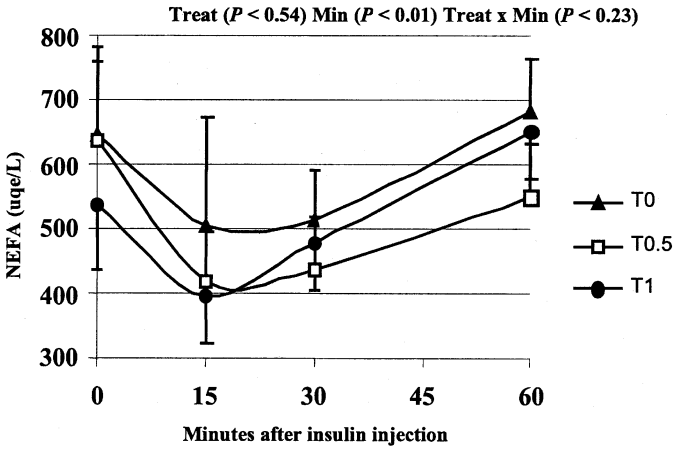

Figure 4. Concentrations of plasma NEFA before and after an intrajugular insulin injection in grazing dairy cows supplemented with 0 (T0), 0.5 (T0.5), and 1 kg/d (T1) of partially hydrogenated oil in early lactation. Samples were taken on d 37 of lactation.

Changes in blood NEFA after the injection of a lipolytic agent were also used in our experiment to evaluate the in vivo adipose tissue lipolytic response after fat feeding. The lack of fat feeding effects on basal NEFA (Table 9) and on the in vivo responses of plasma NEFA to isoproterenol challenge (Table 10) were in accordance with the absence of variations in the body parameters (Figure 2; Table 8) and suggested that lipid mobilization was not reduced by saturated fat feeding in grazing dairy cows in early lactation. These results were also observed in two additional early-lactation trials in grazing dairy cows when corn grain was isoenergetically replaced by the same source of fat (0.7 kg/d) used here (Salado, 2000) or using saturated calcium salts of FA (0.4 kg/d; Gagliostro, 1998). Lipids rich in polyunsaturated FA increased the β-adrenergic lipolytic responses in adipose tissue and losses in BW and BCS when they were infused in the duodenum of lactating dairy cows in early and midlactation experiments (Gagliostro and Chilliard, 1991b). Unsaturated fats may behave differently from saturated fats in their effects on lipolytic responses in lactating dairy cows.

Plasma Concentration of Hormones

No clear effects of dietary fat have been found on circulating ST in lactating dairy cows (Wu and Huber, 1994). When circulating plasma NEFA are increased due to fat supplementation, a decrease in plasma might be expected (Chilliard, 1993; Casper and Schingoethe, 1989). In the present study, neither plasma NEFA nor ST concentrations were affected by fat feeding (Table 9). In a later experiment in grazing conditions, the same source of fat did not change NEFA or ST concentrations in early lactation (Salado, 2000). Although taking serial samples within a day could unmask differences in ST (or insulin) pulsatility, single measurements of hormone concentrations have also been reported in the literature (Gagliostro et al., 1991; Díaz-Torga et al., 2001), and relevant conclusions have been drawn from this kind of analysis.

Plasma insulin was decreased in T1 cows (Table 9), as reported in other studies where supplemental fat was used (Choi and Palmquist, 1996; Staples et al., 1998). The reasons are not clear, because, in our experiment, supplemental fat did not replace corn grain (Table 1), and concentrate intake was not decreased (Table 4). One possible explanation for the lower plasma insu-

lin observed in T1 was the reduction in WSC intake as the consequence of the lower pasture DMI (Table 4). This fact may have decreased the entry of propionic acid and secretion of pancreatic insulin, although plasma glucose concentration was not affected (Table 9). When saturated fat replaced corn grain in the concentrate of early-lactation grazing cows, pasture intake was not affected, and plasma insulin levels or glucose concentrations remained unchanged (Salado, 2000). Dietary fat had no consistent effects on glucose and insulin concentrations (Chilliard, 1993), and Staples et al. (1998) have suggested that changes in plasma insulin are better explained by the energy status of the cows rather than as a direct effect of fat.

As observed in the present study (Table 9), plasma IGF-I concentration was unaffected in lactating cows that received supplemental fat (Salado, 2000). In early-lactation cows, McGuire et al. (1998) found that plasma IGF-I concentration was positively correlated with energy intake and plasma ST levels. In our experiment, the lack of a significant effect of fat on IGF-I concentrations (Table 9) was consistent with the similar ST levels observed (Table 9) and with the failure of fat to enhance energy intake (Table 4). The effects of fat addition on plasma hormones are poorly understood in grazing dairy cows.

Ruminal Environment and Forage NDF Digestion

Long-chain FA with a high melting point have been reported to be adequate for protecting ruminal microbes from deleterious effects of fat (Chalupa et al., 1986). When supplemental fat has negative effects on ruminal digestion, a reduced VFA production and a lower C2:C3 ratio may be expected (Jenkins, 1993). The partially hydrogenated oil used in this experiment did not affect the average concentration of total rumen VFA or the C2:C3 ratio (Table 11). These results suggest that the elevation of the melting point above the ruminal temperature was effective in preventing any negative action of fat on ruminal digestion as proposed by Chalupa (1986). Dietary fat seems to have no consistent effects on ruminal pH values (Palmquist and Jenkins, 1980) as observed in our experiment (Table 11). Supplemental fat did not reduce the concentrations of PUN (Table 9) or MUN (Table 3) in spite of the lower forage DMI (and hence RDP intake) observed in T1 cows (Table 4). This result was consistent with the lack of effect of fat on ruminal NH_3-N concentrations observed in the cannulated cows (Table 11). It seems probable that the partially hydrogenated oil did not affect ruminal forage CP degradation as reported in other grazing experiments (King et al., 1990a).

Palmquist (1984) noted that supplementation with fat of high melting point does not affect rumen fermentation. In accordance with this finding, parameters of pasture NDF digestion were not affected by fat supplementation (Table 12). These parameters also showed a high rate and extent of ruminal fiber digestion and were consistent with the good quality of the forage offered to cows (Table 2). This fact may also help to explain the high pasture DMI observed in cows in treatment T0 (Table 4). On the other hand, the lack of fat effects on parameters of ruminal digestion reinforces the hypothesis that the lower pasture intake observed in T1 (Table 4) would be better explained by metabolic regulation of intake, as discussed above. In our experiment, the increase in the melting point of oil byproducts seems effective in avoiding the negative effects of fat on rumen fiber digestion.

CONCLUSIONS

The addition of energy in the form of high melting point fat to the concentrate fed to grazing dairy cows in early lactation increased the production of FCM and milk fat secretion when it represented about 11% of the total ME requirements of the cows without decreasing milk protein content or yield. Supplemental fat failed to increase total energy intake after peak of lactation because pasture intake was reduced. Because the negative effects of fat on parameters associated with ruminal NDF digestion were not detected, the lower pasture intake was probably explained by a metabolic regulation of energy intake. Although FCM response was higher and energy intake was apparently not changed, supplemental fat did not increase BW loss or plasma NEFA concentrations. The higher secretion of the FA present in the supplement explained in part the observed response in FCM yield. Concentrations of regulatory hormones or changes in the response to lipolytic or insulin challenges were not sensitive to saturated fat feeding. The partial hydrogenation of a byproduct of the oil industry seemed to be effective in preventing the deleterious effects of fat on ruminal digestion. Future research should study the benefit of supplemental fat to early-lactation cows when it replaces energy-yielding substrates to ruminal microbes in grazing production systems.

ACKNOWLEDGMENTS

The authors gratefully thank P. Fay, M. E. Giuliano, M. Camino, M. Patriarca, and C. Basterrechea for their assistance in laboratory analyses and R. Ballesteros and M. Fasciglione for animal care. Help with milk FA composition was given by A. Rodriguez (INTI CITIL,

Miguelete). We are also grateful to M. Melgarejo and Molinos Río de la Plata for donating the partially hydrogenated oil. This research was supported in part by grants from the Agencia Nacional de Promoción Científica y Tecnológica (PICT 3632 and PICT 97-60), CONICET, and by Molinos Río de la Plata. We also thank NIDDK's National Hormone and Pituitary Program and Dr. A. F. Parlow for the bGH radioimmunoassay kit and the anti IGF I antiserum. The English was revised by Dr. Mike H. Wade and Dr. Pablo Corva.

REFERENCES

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis. Vol. I. 15th ed. AOAC, Arlington, VA.

Brumby, P. E., J. E. Storry, J. A. Bines, and R. J. Fulford. 1978. Utilization of energy for maintenance and production in dairy cows given protected tallow during early lactation. J. Agric. Sci. 91:151–159.

Casper, D. P., and D. J. Schingoethe. 1989. Model to describe and alleviate milk protein depression in early lactation dairy cows fed a high fat diet. J. Dairy Sci. 72:3327–3335.

Chalupa, W., B. Vecchiarelli, A. Elser, D. S. Kronfeld, D. Sklan, and D. L. Palmquist. 1986. Ruminal fermentation in vivo as influenced by long-chain fatty acids. J. Dairy Sci. 69:1293–1301.

Chilliard Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: A review. J. Dairy Sci. 76:3897–3931.

Christie, W. W. 1981. The effects of diets and other factors on the lipid composition of ruminant tissue and milk. Pages 193–226 in Lipid Metabolism in Ruminant Animals. W. W. Christie, ed. Pergamon Press, New York.

Choi, B., and D. L Palmquist. 1996. High fat diets increased plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. J. Nutr. 126:2913–2919.

Díaz-Torga, G. S., M. E. Mejía, A. González-Iglesias, D. Becú-Villalobos, and I. M. Lacau-Mengido. 2001. Metabolic cues for puberty onset in free grazing Holstein heifers naturally infected with nematodes. Theriogenology 56:111–122.

Gagliostro, G. A. 1998. Suplementación con lípidos protegidos en vacas lecheras en lactancia temprana: Efectos sobre la producción, la composición de la leche, la concentración de metabolitos plasmáticos y la respuesta a estímulos hormonales. Rev. Arg. Prod. Anim. 18(2):85–100.

Gagliostro, G. A., and Y. Chilliard. 1991a. Duodenal rapeseed oil infusion in early and midlactation cows. 2. Voluntary intake, milk production and composition. J. Dairy Sci. 74:499–509.

Gagliostro, G. A., and Y. Chilliard. 1991b. Duodenal rapeseed oil infusion in early and midlactation cows. 4. *In vivo* and *in vitro* adipose tissue lipolytic responses. J. Dairy Sci. 74:1830–1843.

Gagliostro, G. A., Y. Chilliard, and M. J. Davicco. 1991. Duodenal rapeseed oil infusion in early and mid lactation cows. 3. Plasma hormones and mammary apparent uptake of metabolites. J. Dairy Sci. 74:1893–1903.

Garnsworthy, P. C. 1997. Fats in dairy cow diets. Pages 87–103 in: Recent Advances in Animal Nutrition. P. C. Garnsworthy and D. J. A. Cole, eds. University of Nottingham, UK.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379, ARS-USDA, Washington, DC.

Grummer, R. R., M. L. Hatfield, and M. R. Dentine. 1990. Acceptability of fat supplements in four dairy herds. J. Dairy Sci. 73:852–857.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851–3863.

King, K. R., C. R. Stockdale, and T. E. Trigg. 1990a. Influence of high energy supplements containing fatty acids on the productivity of pasture-fed dairy cows. Aust. J. Exp. Agric. 30:11–16.

King, K. R., C. R. Stockdale, and T. E. Trigg. 1990b. Acceptability of high fat grain mixtures offered to grazing dairy cows. Aust. J. Exp. Agric. 30:595–597.

Komaragiri, M., D. P. Casper, and R. A. Erdman. 1998. Factors affecting body tissue mobilization in early lactation dairy cows. 2. Effect of dietary fat on mobilization of body fat and protein. J. Dairy Sci. 81:169–175.

Lacau-Mengido, I. M., M. E. Mejía, G. S. Díaz-Torga, A. González-Iglesias, N. Formía, C. Libertun, and D. Becú-Villalobos. 2000. Endocrine studies in ivermectin-treated heifers from birth to puberty. J. Anim Sci. 78:817–824.

McGuire, M. A., D. A. Dwyer, D. E. Bauman, and D. E. Smith. 1998. Insulin-like growth factors in plasma and afferent mammary lymph of lactating cows deprived of feed or treated with bovine somatotropin. J. Dairy Sci. 81:950–957.

Mertens, D. R. 1994. Regulation of forage intake. Pages 59–114 in Forage Quality, Evaluation, and Utilization. G. C. Fahey, Jr., ed. Am. Soc. of Agronomy, Inc., Madison, WI.

Minson D. J. 1990. Forage in Ruminant Nutrition. Academic Press, Inc., London.

Morris, D. L. 1948. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107:254–255.

Muller, L. D., and S. L. Fales. 1998. Supplementation of cool-season grass pastures for dairy cattle. Pages 335–350 in Grass for Dairy Cattle. J. H. Cherney and D. J. R. Cherney, ed. CAB International, Wallingford, UK.

National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Natl. Acad. Sci., Washington, DC.

Ørskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb. 92:499–503.

Palmquist, D. L., and T. C. Jenkins. 1980. Fat in lactation rations. Review. J. Dairy Sci. 63:1–14.

Palmquist, D. L. 1984. Use of fats in diets for lactating dairy cows. Pages 357–381 in Fats in Animal Nutrition. J. Wiseman, ed. Butterworths, London.

Pantoja, J., J. L. Firkins, and M. L. Eastridge. 1995. Site of digestion and milk production by cows fed fats differing in saturation, esterification, and chain length. J. Dairy Sci. 78:2247–2258.

Rodriguez, L. A., C. C. Stallings, J. H. Herbein, and M. L. McGilliard. 1997. Effect of degradability of dietary protein and fat on ruminal, blood, and milk components of Jersey and Holstein cows. J. Dairy Sci. 80:353–363.

Salado, E. E. 2000. Reemplazo de grano de maíz por lípidos protegidos en vacas lecheras en pastoreo y en inicio de lactancia. M.S. Thesis. Facultad de Ciencias Agrarias, Universidad Nacional de Mar de Plata, Argentina. 101 pp.

SAS User's Guide: Statistics. 1996. SAS Inst., Inc., Cary, NC.

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. Br. J. Nutr. 32:199–208.

Schingoethe, D. J., and D. P. Casper. 1991. Total lactational response to added fat during early lactation. J. Dairy Sci. 74:2617–2622.

Schroeder, G. F., and G. A. Gagliostro. 2000. Fish meal supplementation to grazing dairy cows in early lactation. J. Dairy Sci. 83:2899–2906.

Staples, C. R., J. M. Burke, and W. W. Thatcher. 1998. Influence of supplemental fats on reproductive tissue and performance of lactating cows. J. Dairy Sci. 81:856–871.

Tilley, J. M. K., and R. A. Terry. 1963. A two stage technique for the in vitro digestion of forage crops. J. Br. Grassland Soc. 18:104.

Wu, Z., and J. T. Huber. 1994. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: A review. Livest. Prod. Sci. 39:141–155.