Feeding Rumen-Inert Fats Differing in Their Degree of Saturation Decreases Intake and Increases Plasma Concentrations of Gut Peptides in Lactating Dairy Cows

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ABSTRACT

Our objective was to determine the effect of feeding rumen-inert fats differing in their degree of saturation on dry matter intake (DMI), milk production, and plasma concentrations of insulin, glucagon-like peptide 1 (7-36) amide (GLP-1), glucose-dependent insulino-tropic polypeptide (GIP), and cholecystokinin (CCK) in lactating dairy cows. Four midlactation, primiparous Holstein cows were used in a 4 × 4 Latin square experiment with 2-wk periods. Cows were fed a control mixed ration *ad libitum*, and treatments were the dietary addition (3.5% of ration dry matter) of 3 rumen-inert fats as sources of mostly saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA). Daily DMI, milk yield, and composition were measured on the last 4 d of each period. Jugular vein blood was collected every 30 min over a 7-h period on d 12 and 14 of each period for analysis of plasma concentrations of hormones, glucose, and nonesterified fatty acids. Feeding fat decreased DMI, and the decrease tended to be greater for MUFA and PUFA compared with SFA. Plasma concentration of GLP-1 increased when fat was fed and was greater for MUFA and PUFA. Feeding fat increased plasma glucose-dependent insulino-tropic polypeptide and CCK concentrations and decreased plasma insulin concentration. Plasma CCK concentration was greater for MUFA and PUFA than for SFA and was greater for MUFA than PUFA. Decreases in DMI in cows fed fat were associated with increased plasma concentrations of GLP-1 and CCK and a decreased insulin concentration. The role of these peptides in regulating DMI in cattle fed fat requires further investigation.

Key words: gut peptide, dietary fat, saturation, intake

INTRODUCTION

Dry matter intake, and particularly energy intake, is a critical determinant of milk production. When DMI is limited in high-producing dairy cows, increasing the NE_L concentration of the diet is one approach that can improve energy intake. The addition of fat is a common practice in the dairy industry; however, increasing the concentration of fat in the diet often decreases DMI (Allen, 2000). The response of DMI to dietary fat varies because of a number of factors, including the stage of lactation, basal diet, and amount and type of fat fed (Firkins and Eastridge, 1994). A number of studies have shown that the type of fat (e.g., FFA or triglycerides) and degree of saturation determine the extent that dietary fat reduces DMI (Schauff and Clark, 1992; Benson et al., 2001; Litherland et al., 2005). The mechanisms by which dietary fat reduces DMI are not completely understood, but gut peptides such as glucagon-like peptide 1 (7-36) amide (GLP-1) and cholecystokinin-8 (CCK), among others, have been postulated as mediators in the regulation of appetite and feed intake in lactating dairy cows (Choi et al., 2000; Ingvarsen and Andersen, 2000; Benson and Reynolds, 2001).

Glucose-dependent insulino-tropic peptide (GIP) and GLP-1 are peptides of gut origin that increase the secretion of insulin in nonruminants under certain conditions (Holst, 1997), thus potentially regulating DMI via an increase in insulin concentration and effects on neuropeptide Y in the hypothalamus (Gale et al., 2004). Effects of GIP on insulin secretion are both direct, through stimulation of the β -cells of the pancreas (Knapper et al., 1996), and indirect, through positive effects on GLP-1 secretion from the L-cells of the distal small intestine (Damholt et al., 1998). Glucagon-like peptide-1 (7-36) amide is also a potential regulator of DMI through inhibitory effects on gut motility and direct effects on the hypothalamus (Shimizu et al., 1987; Turton et al., 1996; Holst, 1997). Because of the digestive and physiological differences between ruminants and nonruminants with regard to nutrient absorption and metabolism, the role of GIP and GLP-1 in the regulation of insulin and appetite in dairy cows is not clearly

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understood. However, a reduction in gut motility caused by the action of GLP-1 could increase rumen fill and physically restrict feed intake. In lactating dairy cows, abomasal infusion of fat increased plasma concentration of GLP-1 and decreased DMI (Benson and Reynolds, 2001; Litherland et al., 2005). Effects of dietary or intragastrically infused fat on the secretion or plasma concentration of GIP have not been reported for cattle, but in preruminant goats, feeding full-fat milk or milk fat increased plasma GIP concentration (Martin et al., 1993). In nonruminants, dietary fat stimulates GIP secretion, but the effect on plasma concentration is more pronounced when dietary fat is consumed with glucose (Knapper et al., 1996).

As with GLP-1, CCK may decrease DMI by decreasing gut motility (Kumar et al., 2004). Although CCK plays a role in the regulation of meal size, total DMI is often not reduced in either nonruminants (Moran, 2004) or cattle (Choi et al., 2000) because of a corresponding increase in the number of meals. Effects of dietary fat on plasma concentration of CCK in ruminants are equivocal. Choi and Palmquist (1996) reported a CCK concentration response in dairy cows fed high-fat diets [calcium salts of palm fatty acids (FA); 9% of DMI], but the effects of including smaller amounts of dietary fat were not significant. In contrast, feeding canola oil (1 kg/d) increased plasma CCK concentration but did not reduce DMI, whereas abomasal infusion of the same amount of canola oil both reduced DMI and increased plasma CCK concentration (Chelikani et al., 2004). In other studies, infusion of fat (vegetable oil) into the abomasum decreased DMI but did not increase plasma CCK concentration (Benson and Reynolds, 2001; Litherland et al., 2005). These observations suggest that the DMI depression in cows fed supplemental fats may be mediated through increased secretion of these gut peptides and subsequent effects on gut motility, insulin secretion, and the hypothalamus. Therefore, the objective of the present study was to determine the extent to which the degree of saturation of supplemental rumen-inert fat would affect DMI and plasma concentrations of GLP-1, CCK, GIP, and insulin in lactating dairy cows.

MATERIALS AND METHODS

Animals and Diets

All animal procedures were approved by the Agricultural Animal Care and Use Committee of The Ohio State University. For 4 wk before the start of the study, 4 primiparous Holstein cows (averaging 614 kg of BW, 165 ± 9 DIM, and 34.4 ± 0.7 kg/d of milk yield at the

Table 1. Formulation and composition (% of DM basis) of the control diet and diets supplemented with fats containing mostly saturated fatty acids (SFA), mostly monounsaturated fatty acids (MUFA), and mostly polyunsaturated fatty acids (PUFA)

Item	Control	SFA	MUFA	PUFA
Ingredient				
Corn silage	38	38	38	38
Alfalfa silage	17	17	17	17
Corn meal	14.36	10.01	10.82	11.11
Soybean meal, 44% CP	15.57	16.28	16.16	16.10
Soybean hulls	11.10	11.10	11.10	11.10
Vegetable oil	0.508	0.508	0.508	0.508
Energy Booster 100 Advance ¹	—	3.571	—	—
EnerG-II ²	—	—	3.912	—
Calcium salts of soybean oil ³	—	—	—	4.154
Dicalcium phosphate	0.432	0.480	0.460	—
Monosodium phosphate	—	—	—	0.40
Limestone	1.45	1.42	0.43	0.00
Magnesium oxide	0.102	0.102	0.102	0.102
Trace mineral salt ⁴	0.716	0.716	0.712	0.712
Selenium, 198 mg/kg	0.163	0.163	0.163	0.163
Zinc sulfate	0.005	0.005	0.005	0.005
Zinpro 100 ⁵	0.005	0.005	0.005	0.005
Copper sulfate	0.005	0.005	0.005	0.005
Biotin	0.488	0.488	0.488	0.488
Vitamin A, 30,000 IU/g	0.018	0.018	0.018	0.018
Vitamin D, 3,000 IU/g	0.061	0.061	0.061	0.061
Vitamin E, 44.08 IU/g	0.071	0.071	0.071	0.071
Composition				
CP	16.1	16.1	15.8	15.9
NDF	34.8	34.8	35.8	34.1
Ash	6.8	6.7	6.9	6.6
Total fatty acids	2.34	5.14	5.15	5.08
Starch	24.8	21.5	21.5	22.5

¹Energy Booster 100 Advance (MS Specialty Nutrition, Dundee, IL).

²Calcium salts of palm distillate (Virtus Nutrition, Fairlawn, OH).

³Calcium salts of soybean oil fatty acids provided by Virtus Nutrition (Fairlawn, OH).

⁴Containing zinc, 3,500 ppm; manganese, 2,800 ppm; copper, 400 ppm; and iodine, 70 ppm.

⁵Zinc (100 mg/kg) and methionine (200 mg/kg) complex (Zinpro, Eden Prairie, MN).

start of the trial) were fed a control diet (Table 1) of a mixed ration formulated to meet nutrient requirements (NRC, 2001). The formulation of the control diet was based on the ration that was fed to the cows immediately before they were used for the present study. Cows were housed in individual tie stalls with water cups and were milked at 0130 and 1300 h in the herd parlor. Daily rations were provided at 0800 h, and cows were fed ad libitum (10% refusal). Ration composition was adjusted weekly for changes in component DM concentration.

Treatments and Experimental Design

Treatments were the dietary inclusion of rumen-inert fat sources differing in their degree of saturation in a 4 × 4 balanced Latin square design with 2-wk periods. Treatments were the control diet (without supplement-

Table 2. Fatty acid composition (g/100 g of total fatty acids) of the control diet and diets supplemented with rumen-inert fats containing mostly saturated fatty acids (SFA), mostly monounsaturated fatty acids (MUFA), or mostly polyunsaturated fatty acids (PUFA)

FA	Control	SFA	MUFA	PUFA
12:0	0.76	0.36	0.36	0.41
14:0	0.39	1.86	0.80	0.28
16:0	16.67	25.61	33.35	13.82
16:1	0.06	0.43	0.29	0.26
18:0	3.82	30.22	4.21	5.03
18:1 <i>cis</i> -9	18.94	11.90	28.75	20.73
18:1 <i>cis</i> -11	1.22	0.75	0.91	1.30
18:2	40.90	19.20	23.05	45.14
18:3 n-6	0.51	0.25	0.23	0.24
18:3 n-3	7.99	3.79	3.70	8.13
20:0	0.41	0.21	0.23	0.42
20:2	0.15	0.09	0.12	0.15
22:0	0.45	0.27	0.23	0.40
22:5	0.04	0.02	0.02	0.02
24:0	0.54	0.27	0.27	0.32
Others	7.14	4.77	3.48	3.35

tal fat) or 3 diets containing 1 of 3 supplemental rumen-inert fats: mostly (87%) saturated FA [SFA; prilled hydrogenated FFA from tallow; Energy Booster 100 Advance (EB), MS Specialty Nutrition, Dundee, IL], mostly (36%) monounsaturated FA (MUFA) from palm distillate (EnerG-II, Virtus Nutrition, Fairlawn, OH), and mostly (58%) polyunsaturated FA (PUFA; calcium salts of soybean oil FA [CaSoy], Virtus Nutrition). Dietary fat was added at 3.5% of ration DM, replacing ground corn, and diets were balanced for CP and calcium to account for the removal of corn and the addition of calcium salts (Table 1). The amount of supplemental fat fed was based on the amounts infused abomasally in previous studies in which a depression in DMI and an increase in gut peptide concentrations were observed (Benson and Reynolds, 2001).

Measurements

Dry matter intake and milk yield and composition were measured for the last 4 d of each period, as described by Beckman and Weiss (2005). Feed samples were taken weekly and pooled for each period and analyzed for DM (100°C), ash (600°C), Kjeldahl N, starch, NDF, and total FA (Table 1), as described by Beckman and Weiss (2005). The FA profile of the diets fed is presented in Table 2. A milk sample preserved with bronopol was stored at 2°C until analyzed for fat, lactose, and protein concentrations using infrared analysis (DHI Cooperative, Columbus, OH). Milk fat from samples taken from the last 2 milkings of each period was separated by centrifugation for 30 min (20,000 × *g* at 4°C) and stored frozen at –80°C until constituent FA were converted to methyl esters and relative concentra-

tions determined by GLC, as described previously (Kramer et al., 1997; Reveneau et al., 2005).

Blood Sampling and Analysis

Jugular vein blood was sampled on d 12 and 14 of each period using catheters (Huntington et al., 1989) implanted on d 11 of each period. Blood samples (10 mL) were taken at 30-min intervals from 0700 to 1400 h, except at 1300 h when cows were in the milking parlor. Blood samples were immediately transferred to tubes containing solutions of disodium EDTA and benzamidine HCl (1.6 and 4.7 mg/mL of blood, respectively) and placed on ice. After centrifugation for 30 min (1,800 × *g* and 4°C), blood was aliquoted into individual polypropylene tubes for each hormone analysis to be performed, flash-frozen using liquid N₂, and stored at –80°C until analyzed. For CCK, glucose, and NEFA analysis, 3 sequential plasma samples were composited in a tube containing 500 Kallikrein-inhibitor units of dried aprotinin (Trasylol; Bayer AG, Leverkusen, Germany) per milliliter of plasma added (Benson and Reynolds, 2001) and flash-frozen after each plasma addition.

Hormone and Metabolite Analysis

Concentrations of insulin, GLP-1, GIP, and CCK were measured using double-antibody RIA. The intraassay coefficients were less than 10% for insulin, 6% for GLP-1, 11% for GIP, and 4% for CCK. Plasma insulin concentration was measured using an equilibrium assay based on the assay described by Reynolds et al. (1989). Sensitivity of the insulin assay (the concentration at which bound counts were 90% of binding for the zero standard) was 0.0027 pmol/tube. Plasma GLP-1 concentration was measured using a disequilibrium assay based on the assay described by Benson and Reynolds (2001). Sensitivity of the GLP-1 assay was 0.001 pmol/tube. Plasma GIP concentration was measured much as described by Morgan et al. (1978) using a disequilibrium assay with 24- and 48-h incubations before addition of labeled GIP (#T-027-02; Phoenix Pharmaceutical, Inc., Belmont, CA) and secondary antibody (goat anti-rabbit IgG, AB21; Chemicon International, Temecula, CA), respectively. The anti-human GIP (Rab-027-02; Phoenix Pharmaceutical, Inc.) has 100% cross-reactivity with human and porcine GIP. Serial addition of bovine plasma displaced labeled GIP linearly, with a slope similar to the displacement by porcine standards. Sensitivity of the GIP assay was 0.025 pmol/tube. Plasma CCK concentration was measured in extracted plasma using a commercial disequilibrium assay (Euro-Diagnostica, Malmö, Sweden), as described by Benson and

Reynolds (2001). Plasma (500 μ L) was extracted with 96% ethanol (1 mL) and then centrifuged at $1,700 \times g$ for 15 min at 4°C. The supernatant was decanted to another tube, evaporated in a freeze-dryer overnight, and then reconstituted in assay buffer before analysis. Sensitivity of the CCK assay was 0.0006 pmol/tube. Plasma glucose concentration was measured using an enzymatic assay (#1070 Glucose Trinder Assay; Stanbio Laboratory, Boerne, TX). Plasma NEFA concentration was measured using an enzymatic assay (Wako Chemicals USA, Inc., Richmond, VA) as described by Johnson and Peters (1993).

Statistical Analysis

Because of a mistake in feeding the CaSoy diet in the first period, data from one animal fed this diet were excluded from the statistical analysis. Thus, one observation was missing from the 16 possible cow and period combinations. Data for DMI and milk production and composition were averaged for each cow-period ($n = 15$) and analyzed using the MIXED procedure of SAS (SAS Institute, 2000) with a model testing the random effects of animal and period and the fixed effect of diet. Cows were sampled on 2 d at the end of each period to account for variation in plasma concentrations of hormones over time and to provide a more robust measurement of any postprandial responses. Hormone and metabolite concentrations were averaged for each time point across d 12 and 14 and analyzed as repeated measures over sampling times using the MIXED procedure of SAS (SAS Institute, 2000). The model used tested the random effects of animal and period and the fixed effects of diet and sampling time. Data were analyzed using the structure giving the best fit based on Akaike's information criterion (Littell et al., 1996). Treatment means were separated using orthogonal contrasts to partition treatment sums of squares into the following comparisons: control vs. supplemental fat (control vs. EB, EnerG-II, and CaSoy), SFA vs. unsaturated fat (EB vs. EnerG-II and CaSoy) and MUFA vs. PUFA (EnerG-II vs. CaSoy).

RESULTS

DMI, Milk Yield, and Milk Composition

Feeding supplemental fat decreased ($P < 0.05$) DMI (Table 3), and the effect was numerically greater ($P < 0.12$) for MUFA and PUFA (Table 3) than for SFA. There was no overall effect of diet on estimated ME intake or milk yield, but MUFA and PUFA tended ($P < 0.11$) to decrease milk yield compared with SFA. The milk fat percentage and yield were affected by feeding fat ($P < 0.06$ and $P < 0.04$, respectively) because of a

decrease when PUFA were fed ($P < 0.09$ compared with MUFA) and an increase when SFA were fed ($P < 0.03$ compared with MUFA and PUFA; Table 3). There was no effect of diet on milk protein concentration and yield, but feeding MUFA and PUFA decreased milk lactose percentage and yield ($P < 0.05$ and $P < 0.06$, respectively) compared with SFA (Table 3).

Milk FA Composition

Feeding supplemental fat decreased ($P < 0.01$) relative concentrations of a number of shorter- and medium-chain-length SFA (8:0, 10:0, 12:0, 14:0, 15:0) in milk fat, and the effect was more pronounced for 8:0, 10:0, and 14:0 when MUFA and PUFA were fed compared with SFA ($P < 0.03$ or less; Table 4). In contrast, feeding SFA increased ($P < 0.01$) the relative concentration of 16:0 compared with MUFA and PUFA, and feeding PUFA decreased ($P < 0.01$) 16:0 concentration relative to MUFA. Feeding supplemental fats increased ($P < 0.05$ or less) the relative concentrations of *trans*-11 18:1, total *trans*-18:1, and *trans*-10, *cis*-12 18:2, and the increase was greater for MUFA and PUFA compared with SFA ($P < 0.01$), and for PUFA compared with MUFA ($P < 0.10$ or less). Relative concentrations of total conjugated linoleic acid (CLA, $P < 0.03$) and total *trans*-18:1 ($P < 0.01$) were greater when MUFA and PUFA were fed compared with SFA, and for PUFA compared with MUFA ($P < 0.01$). These differences in total CLA and total *trans*-18:1 were largely due to differences in the concentrations of *cis*-9, *trans*-11 18:2 ($P < 0.01$) and *trans*-11 18:1 ($P < 0.01$), respectively. The concentration of 18:2 was increased ($P < 0.04$) by feeding MUFA and PUFA compared with SFA, and was greater ($P < 0.04$) when PUFA were fed compared with MUFA. Finally, the concentration of *cis*-9 18:1 was increased ($P < 0.04$) when MUFA were fed compared with PUFA.

Plasma Concentrations of Hormones and Metabolites

Plasma glucose concentration and all the hormones measured varied over time of sampling ($P < 0.03$; Table 5 and Figure 1), but no significant time $\times$ treatment interactions were observed ($P > 0.25$). For the hormones, the effect of time was due to a postprandial increase in plasma concentration. In contrast, plasma glucose concentration decreased after feeding and then returned to the initial concentration by the end of sampling (Figure 1). The concentration of GLP-1 was increased ($P < 0.01$) by feeding supplemental fat (Table 5), and the response was greater for MUFA and PUFA compared with SFA ($P < 0.04$). There was no difference ($P = 0.55$) in GLP-1 concentration for cows fed MUFA

Table 3. Dry matter intake and milk yield, composition, and component yield in midlactation dairy cows fed rumen-inert fats containing mostly saturated fatty acids (SFA), mostly monounsaturated fatty acids (MUFA), or mostly polyunsaturated fatty acids (PUFA)

Item	Diet				SEM	$P <^1$		
	Control-1	SFA-2	MUFA-3	PUFA-4		1 vs. 2-4	2 vs. 3, 4	3 vs. 4
DMI, kg/d	23.8	23.1	22.1	22.0	1.1	0.05	0.12	0.73
ME intake, MJ/d	249	254	244	248	11	0.98	0.25	0.64
Milk, kg/d	36.9	37.3	35.8	34.8	1.4	0.39	0.11	0.44
Fat, %	3.37	3.86	3.32	2.61	0.25	0.67	0.03	0.08
Protein, %	3.08	3.06	3.04	3.01	0.05	0.45	0.49	0.62
Lactose, %	4.82	4.83	4.72	4.76	0.07	0.20	0.05	0.36
Fat, g/d	1,249	1,436	1,184	911	0.1	0.53	0.02	0.09
Protein, g/d	1,127	1,141	1,081	1,048	0.1	0.41	0.13	0.55
Lactose, g/d	1,769	1,801	1,679	1,659	0.1	0.36	0.07	0.77

¹Probability corresponding to the hypothesis of no effect of supplemental fat (1 vs. 2, 3, 4), no difference between saturated and unsaturated fat supplements (2 vs. 3, 4), and no difference between MUFA and PUFA (3 vs. 4).

vs. PUFA (Table 5 and Figure 1). Feeding fat increased plasma GIP concentration ($P < 0.01$), but the increase was numerically greater ($P < 0.13$) for SFA compared with MUFA and PUFA (Table 5 and Figure 1). Plasma concentration of insulin decreased ($P < 0.04$) when MUFA and PUFA were fed (Table 5 and Figure 1). Feeding fat also increased CCK concentration ($P < 0.01$), and the effect was greater for PUFA and MUFA compared with SFA ($P < 0.02$) and for MUFA compared with PUFA ($P < 0.04$; Table 5 and Figure 1). Plasma

glucose concentration (Table 5 and Figure 1) tended to decrease ($P < 0.11$) when fat was fed. Plasma concentration of NEFA increased ($P < 0.01$) when fat was fed, and the effect was greater ($P < 0.01$) for MUFA and PUFA than for SFA.

DISCUSSION

DMI, Milk Yield, and Milk Composition

In the present study, the decrease in DMI caused by feeding fat followed the same pattern observed in

Table 4. Milk fatty acid (FA) composition (g/100 g) in midlactation dairy cows fed rumen-inert fats containing mostly saturated fatty acids (SFA), mostly monounsaturated fatty acids (MUFA), or mostly polyunsaturated fatty acids (PUFA)

FA	Diet				SEM	$P <^1$		
	Control-1	SFA-2	MUFA-3	PUFA-4		1 vs. 2-4	2 vs. 3, 4	3 vs. 4
6:0	1.42	1.40	1.11	0.88	0.29	0.32	0.21	0.57
8:0	1.18	0.97	0.62	0.52	0.13	0.01	0.03	0.58
10:0	3.03	2.13	1.26	1.14	0.26	0.01	0.02	0.75
12:0	3.34	2.08	1.65	1.63	0.28	0.01	0.21	0.97
14:0	12.76	9.93	7.99	8.11	0.56	0.01	0.02	0.85
15:0	1.33	0.92	0.77	0.72	0.12	0.01	0.14	0.71
16:0	28.42	31.36	30.41	20.87	1.06	0.50	0.01	0.01
16:1	0.50	1.04	1.12	0.66	0.49	0.46	0.81	0.55
17:0	0.50	0.87	0.29	0.42	0.20	0.92	0.08	0.70
18:0	7.73	9.04	8.59	7.70	1.09	0.59	0.53	0.61
18:1 <i>trans</i> -10	ND	0.11	0.24	ND	0.14	0.45	0.96	0.21
18:1 <i>trans</i> -11	2.16	3.59	5.23	13.05	0.99	0.01	0.01	0.01
18:1 total <i>trans</i>	2.40	3.74	5.92	17.30	1.76	0.02	0.01	0.01
18:1 <i>cis</i> -9	19.67	22.09	26.07	19.38	1.93	0.15	0.75	0.04
18:1 <i>cis</i> -11	1.44	0.74	0.65	1.33	0.43	0.32	0.66	0.34
18:2	2.86	2.32	2.52	3.08	0.16	0.23	0.04	0.05
18:2 <i>cis</i> -9, <i>trans</i> -11	0.60	0.46	0.60	2.50	0.35	0.16	0.03	0.01
18:2 <i>trans</i> -10, <i>cis</i> -12	0.01	0.02	0.03	0.05	0.01	0.05	0.01	0.10
Total CLA ²	0.65	0.51	0.67	2.60	0.34	0.14	0.03	0.01
18:3 n-3	0.48	0.391	0.50	0.41	0.08	0.63	0.57	0.49
Others	2.96	2.28	2.44	7.02	1.50	0.60	0.23	0.08

¹Probability corresponding to the hypothesis of no effect of supplemental fat (1 vs. 2, 3, 4), no difference between saturated and unsaturated fat supplements (2 vs. 3, 4), and no difference between MUFA and PUFA (3 vs. 4).

²Conjugated linoleic acid.

Table 5. Hormone and metabolite concentrations in midlactation dairy cows feed 3 rumen-inert fat sources differing in degree of saturation: mostly saturated fatty acids (SFA), mostly monounsaturated fatty acids (MUFA), and mostly polyunsaturated fatty acids (PUFA)

Item ¹	Diet				SEM	<i>P</i> < ²			
	Control-1	SFA-2	MUFA-3	PUFA-4		Time	1 vs. 2-4	2 vs. 3, 4	3 vs. 4
GLP-1, nM	0.028	0.030	0.033	0.032	0.002	0.01	0.01	0.04	0.53
GIP, nM	0.499	0.590	0.570	0.534	0.063	0.03	0.01	0.13	0.19
Insulin, nM	0.188	0.187	0.160	0.158	0.027	0.01	0.07	0.04	0.89
CCK, pM	10.0	12.6	15.9	13.8	1.57	0.01	0.01	0.02	0.04
Glucose, mM	3.43	3.35	3.19	3.28	0.10	0.01	0.11	0.28	0.43
NEFA, mM	0.089	0.097	0.137	0.126	0.012	0.30	0.01	0.01	0.34

¹GLP-1 = glucagon-like peptide 1 (7-36) amide; GIP = glucose-dependent insulinotropic polypeptide; CCK = cholecystokinin.

²Probability corresponding to the hypothesis of no effect of sampling time within day (time), no effect of supplemental fat (1 vs. 2, 3, 4), no difference between saturated and unsaturated fat supplements (2 vs. 3, 4), and no difference between MUFA and PUFA (3 vs. 4).

previous studies (Drackley et al., 1992; Choi and Palmquist, 1996; Benson et al., 2001), in which feeding or abomasally infusing fat decreased DMI of lactating dairy cows, and a more pronounced decrease was observed when unsaturated fats were provided. The general effect on milk yield of feeding fat was not signifi-

cant, as observed previously when fat was fed for the same period of time (Choi and Palmquist, 1996) or infused into the abomasum for 7 d (Benson et al., 2001); however, there was a tendency for milk yield to decrease when MUFA or PUFA were fed. This numeric decrease in milk yield could be related to the decrease in DMI

Figure 1. Plasma concentrations of glucose, glucagon-like peptide 1 (7-36) amide (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), NEFA, cholecystokinin (CCK), and insulin during a 7-h sampling period for midlactation dairy cows fed a control diet (◇) or a diet with supplemental fat containing either mostly saturated (□), mostly monounsaturated (×), or mostly polyunsaturated (△) fatty acids. Cows were fed immediately after sampling at 0800 h.

observed, although the estimated ME intake was not affected. Cows in the present study were in midlactation, and the production and hormonal responses to supplemental fat may differ in early lactation. Changes in milk FA profile (Table 4) indicate that at least partial biohydrogenation of the MUFA and PUFA fed as calcium salts was occurring in the rumen. The incomplete protection of calcium salts of soybean oil from rumen hydrogenation was described previously (Chouinard et al., 1998; Lundy et al., 2004). Increased relative concentrations of *trans*-11 18:1, *cis*-9, *trans*-11 18:2, *trans*-10, *cis*-12 18:2, total *trans*-18:1, and CLA when MUFA and PUFA were fed reflect their synthesis as intermediates in the biohydrogenation of linoleic acid (*cis*-9, *cis*-12 18:2) to stearic acid, and the synthesis of *cis*-9, *trans*-11 18:2 from *trans*-11 18:1 in the mammary gland (Bauman and Griinari, 2003). An increased ruminal production and absorption of *trans*-10, *cis*-12 18:2 may be responsible for the decrease in milk fat concentration and yield (Table 3) when MUFA and PUFA were fed. Biohydrogenation of *cis*-9, *cis*-12 18:2 in the CaSoy supplement may have reduced the amount of PUFA reaching the small intestine, which is a possible explanation for differences in the responses of gut peptide concentrations observed for the 2 unsaturated FA supplements (MUFA vs. PUFA).

Milk FA concentrations were measured as an indicator of the relative changes achieved in the profile of FA absorbed by feeding the rumen-inert fat supplements used in the present study. The responses observed are typical of other studies in which these or similar fat supplements were fed to lactating dairy cows (for a review see, e.g., Palmquist, 2006). Reductions in the concentration of short- and medium-chain SFA, although relatively small in magnitude (Table 4), are often observed when fats are fed or abomasally infused and the concentrations of longer-chain FA are increased (Drackley et al., 1992; Palmquist, 2006). The increased concentration of 16:0 when SFA and MUFA were fed reflects the content (39 to 50%) of this FA in the fat supplements fed for these treatments. Changes in the relative concentrations of *trans*-18:1 isomers and CLA indicate that the CaSoy product was less protected from ruminal biohydrogenation than the other supplements fed; however, the increased milk fat concentration of *cis*-9, *cis*-12 18:2 indicates that a greater absorption of this FA occurred when PUFA were fed compared with the other treatments. Previous studies have reported the extent of biohydrogenation of dietary FA when the same or similar supplemental fats were fed to ruminants. Although the unsaturated FA in EB can be subjected to biohydrogenation, the unsaturated FA content of this product is typically low (less than 15%; Grummer et al., 1996). Calcium salts of FA can also be subject to

ruminal biohydrogenation, depending on the dissociation constants of their component FA, the type of ration fed, and other factors influencing rumen environment and pH (Sukhija and Palmquist, 1990). In lactating dairy cows fed supplemental EB or calcium salts of palm oil (Harvatine and Allen, 2006), the extent of biohydrogenation of total dietary *cis*-9, *cis*-12 18:2 was 84.5 and 86.6%, respectively, whereas the biohydrogenation of *cis*-9 18:1 was 71.5 and 63.6%, respectively. Lundy et al. (2004) also reported extensive biohydrogenation of unsaturated FA in diets supplemented with calcium salts of soybean oil FA that were fed to lactating dairy cows (77.9% of *cis*-9 18:1 and 92.2% of *cis*-9, *cis*-12 18:2), but they also observed a marked increase in *cis*-9, *cis*-12 18:2 flow to the omasum (from 25 to 39 g/d).

Gut Hormones, Insulin, and Metabolites

In the present study, a postprandial increase in plasma concentrations of insulin, GLP-1, GIP, and CCK was observed after feeding (Figure 1), but the increase was relatively small for GLP-1. A postprandial increase in arterial insulin concentration was reported previously in dairy cows (Choi and Palmquist, 1996; Choi et al., 2000; Benson and Reynolds, 2001). In the study by Benson and Reynolds (2001), an increased insulin concentration accompanied an increase in net VFA and glucose absorption across the portal-drained viscera (Benson et al., 2002), indicating a response of insulin secretion to increased postprandial nutrient absorption and glucose supply. A postprandial increase in the arterial concentration of GLP-1 was not observed by Benson and Reynolds (2001), but in their study, cows were fed daily rations in 3 equal meals and supplemental fat was infused continuously. In the present study, cows were fed their rations and supplemental fat once daily, and numerical increases in plasma GLP-1 and GIP concentrations over the course of the sampling period were less when control diets were fed (Figure 1). The postprandial increase in plasma CCK concentration observed in the present study may be due to increased digesta flow and nutrient absorption from the small intestine, stimulating intestinal K cells to secrete CCK; however, a postprandial increase in plasma CCK concentration was not observed in previous studies in which lactating dairy cows were fed once daily (Choi and Palmquist, 1996; Choi et al., 2000; Chelikani et al., 2004). In the present study, the highest CCK concentration was observed after the afternoon milking at 1300 h (Figure 1), but Samuelsson et al. (1996) observed no effect of milking or feeding on plasma CCK concentration.

Plasma GLP-1 concentration increased when supplemental fat was fed, and the increase was greater when

the fat fed was less saturated, but the responses to MUFA and PUFA fat sources were similar. Our results differ from those of Beysen et al. (2002), who reported that in humans, MUFA are a more potent secretagogue for GLP-1 than are PUFA; however, Thomsen et al. (1999) reported no difference in plasma concentration of GLP-1 in humans after a single meal of a food rich in SFA compared with MUFA. Nevertheless, the length of the treatment period may be important because the response of plasma GLP-1 concentration to an increased intestinal supply of FA may differ over time, that is, an acute (hours) vs. chronic (days) response. The mechanism by which GLP-1 secretion is regulated by dietary FA is not clearly understood; however, Hirasawa et al. (2005) suggested that this regulation is via a G protein-coupled receptor (GPR120) for unsaturated FA in the cells of the small intestine. Damholt et al. (1998) reported that GIP is a secretagogue for GLP-1, but in the present study differences in the response of plasma concentration of GLP-1 to the type of fat fed could not be attributed to changes in plasma GIP concentration. Plasma GIP concentration increased when fat was added to the diet, but there was no significant difference in responses across the fat supplements. In contrast, Thomsen et al. (1999) reported a difference in the response of GIP concentration when FA differing in degree of saturation were fed to humans. In that study, subjects fed MUFA had a greater plasma concentration of GIP than when mostly SFA were fed.

The decrease in insulin concentration observed when supplemental fat was fed was associated with decreased DMI, which would decrease the absorption of insulin secretagogues such as glucose and propionate. There seemed to be no relationship between changes in the concentrations of insulin and the incretin hormones GLP-1 and GIP. In the present study, plasma concentration of glucose was less than 4.5 mM, which is the concentration proposed by Holst (1997) as the threshold for GLP-1 and GIP to stimulate insulin secretion in humans. Faulkner and Martin (1999) reported an increased plasma insulin concentration in lactating sheep when GLP-1 was intravenously infused with glucose, and a plasma glucose concentration of 4.78 mM was observed in the jugular vein, but an increase in insulin concentration was not observed when GLP-1 was infused without glucose and plasma glucose concentration was 3.24 mM.

In contrast to other studies in which fat was added to the diet (Choi and Palmquist, 1996) or infused into the abomasum (Benson and Reynolds, 2001; Litherland et al., 2005), plasma CCK concentration increased with the addition of fat to diets in our study. However, Chelikani et al. (2004) found that abomasal infusion and dietary inclusion of relatively large amounts (1 kg) of

canola oil, which is high in MUFA, increased intestinal CCK mRNA expression and plasma concentrations of CCK in lactating dairy cows. In addition, Harvatine and Allen (2005) reported that feeding lactating dairy cows fats high in unsaturated FA increased plasma CCK concentration compared with feeding a fat high in SFA. These data and the results of the present study indicate that the response of plasma CCK concentration is influenced by the relative amounts of unsaturated FA reaching the small intestine, which is similar to findings in humans (Robertson et al., 2002). Differences in plasma CCK response to supplemental fats provided by postruminal infusion (Benson and Reynolds, 2001; Litherland et al., 2005), compared with the present study in which supplemental fat was fed, may be due to differences in the timing of supplemental FA delivery to the small intestine (constant infusion vs. postprandial increase).

The reason for a greater response of plasma CCK concentration to feeding MUFA vs. PUFA is not clearly understood, but it might be a consequence of ruminal biohydrogenation of the PUFA in CaSoy. Although differences in milk FA profile indicate that rumen biohydrogenation occurred, a better understanding of the profile and amount of FA absorbed by the small intestine is needed to explain our results. In the study by Chelikani et al. (2004), feeding canola oil increased plasma CCK concentration but did not reduce DMI. Our assumption was that the increase in CCK concentration observed was more important for gut function and motility (Kumar et al., 2004) and exocrine pancreatic secretion (Tachibana et al., 1995), which would benefit nutrient digestion and especially fat digestion, and could in part explain why SFA are less digestible than unsaturated FA (Firkins and Eastridge, 1994).

The increase in NEFA concentration when fat was fed may be explained by an increase in fat absorption along with a decrease in insulin concentration, which could allow for more lipolysis in adipose tissue. In the present study, plasma NEFA concentrations were lower in cows fed SFA vs. unsaturated FA, which agrees with the results of studies showing that dietary SFA decrease lipolysis in rats to a greater extent than do dietary PUFA (Awad and Chattopadhyay, 1986).

In conclusion, the decrease in DMI observed when supplemental fat was fed was associated with an increase in plasma concentration of GLP-1. This gut peptide hormone has hypophagic effects, and its concentration was highest when fats high in MUFA and PUFA were fed and DMI was lowest. In present study, we did not observe an increase in plasma insulin concentration when increases in plasma GIP and GLP-1 were observed; however, glucose concentration, which modulates insulin secretion, was always below the threshold

value of 4.5 mM proposed by Holst (1997) as required for these gut peptides to have positive effect on insulin secretion in humans. In contrast to previous studies in our laboratory in which supplemental fat was infused postruminally, in the present study plasma concentration of CCK increased when supplemental fat was fed, and the response of plasma CCK to dietary fat was greatest when the fat source was high in MUFA. Results of the present and previous studies implicate GLP-1, or other cosecreted products of proglucagon processing (e.g., oxyntomodulin or GLP-2), as potential mediators of the effects of dietary fat on DMI in lactating dairy cows. Further research is needed to understand the mechanisms by which dietary FA regulate the secretion of gut peptides, the interactions of these hormones, and their effects on gut function and DMI in dairy cattle.

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REFERENCES

- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- Awad, A. B., and J. P. Chattopadhyay. 1986. Effect of dietary saturated fatty acids on hormone-sensitive lipolysis in rat adipocytes. *J. Nutr.* 116:1088–1094.
- Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. *Annu. Rev. Nutr.* 23:203–227.
- Beckman, J. L., and W. P. Weiss. 2005. Nutrient digestibility of diets with different fiber to starch ratios when fed to lactating dairy cows. *J. Dairy Sci.* 88:1015–1023.
- Benson, J. A., and C. K. Reynolds. 2001. Effects of abomasal infusion of long-chain fatty acids on splanchnic metabolism of pancreatic and gut hormones in lactating dairy cows. *J. Dairy Sci.* 84:1488–1500.
- Benson, J. A., C. K. Reynolds, P. C. Aikman, B. Lupoli, and D. E. Beever. 2002. Effects of abomasal long chain fatty acid infusion on splanchnic nutrient metabolism in lactating dairy cows. *J. Dairy Sci.* 85:1804–1814.
- Benson, J. A., C. K. Reynolds, D. J. Humphries, S. M. Rutter, and D. E. Beever. 2001. Effects of abomasal infusion of long-chain fatty acids on intake, feeding behavior and milk production in dairy cows. *J. Dairy Sci.* 84:1182–1191.
- Beysen, C., F. Karpe, B. A. Fielding, A. Clark, J. C. Levy, and K. N. Frayn. 2002. Interaction between specific fatty acids, GLP-1 and insulin secretion in humans. *Diabetologia* 45:1533–1541.
- Chelikani, P. K., D. R. Glimm, D. H. Keisler, and J. J. Kennelly. 2004. Effects of feeding or abomasal infusion of canola oil in Holstein cows. 2. Gene expression and plasma concentrations of cholecystokinin and leptin. *J. Dairy Res.* 71:288–296.
- Choi, B. R., and D. L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. *J. Nutr.* 126:2913–2919.
- Choi, B. R., D. L. Palmquist, and M. S. Allen. 2000. Cholecystokinin mediates depression of feed intake in dairy cattle fed high fat diets. *Domest. Anim. Endocrinol.* 19:159–175.
- Chouinard, P. Y., V. Girard, and G. J. Brisson. 1998. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with varying unsaturation. *J. Dairy Sci.* 81:471–481.
- Damholt, A. B., A. M. Buchan, and H. Kofod. 1998. Glucagon-like-peptide-1 secretion from canine L-cells is increased by glucose-dependent-insulinotropic peptide but unaffected by glucose. *Endocrinology* 139:2085–2091.
- Drackley, J. K., T. H. Klusmeyer, A. M. Trusk, and J. H. Clark. 1992. Infusion of long-chain fatty acids varying in saturation and chain length into the abomasum of lactating dairy cows. *J. Dairy Sci.* 75:1517–1526.
- Faulkner, A., and P. A. Martin. 1999. Insulin secretion and intestinal peptides during lactation in sheep. *J. Dairy Res.* 66:45–52.
- Firkins, J. L., and M. L. Eastridge. 1994. Assessment of the effects of iodine value on fatty acid digestibility, feed intake, and milk production. *J. Dairy Sci.* 77:2357–2366.
- Gale, S. M., V. D. Castracane, and C. S. Mantzoros. 2004. Energy homeostasis, obesity and eating disorders: Recent advances in endocrinology. *J. Nutr.* 134:295–298.
- Grummer, R. R., K. Slark, S. J. Bertics, M. L. Luck, and J. A. Barmore. 1996. Soybeans versus animal sources of rumen-undegradable protein and fat for early lactation dairy cows. *J. Dairy Sci.* 79:1809–1816.
- Harvatine, K. J., and M. S. Allen. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. *J. Dairy Sci.* 88:4018–4027.
- Harvatine, K. J., and M. S. Allen. 2006. Fat supplements affect fractional rates of ruminal biohydrogenation and passage in dairy cows. *J. Nutr.* 136:677–685.
- Hirasawa, A., K. Tsumaya, T. Awaji, S. Katsuma, T. Adachi, M. Yamada, Y. Sugimoto, S. Miyazaki, and G. Tsujimoto. 2005. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.* 11:90–94.
- Holst, J. J. 1997. Enteroglucagon. *Annu. Rev. Physiol.* 59:257–271.
- Huntington, G. B., C. K. Reynolds, and B. H. Stroud. 1989. Techniques for measuring blood flow in splanchnic tissues of cattle. *J. Dairy Sci.* 72:1583–1595.
- Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. *J. Dairy Sci.* 83:1573–1597.
- Johnson, M. M., and J. P. Peters. 1993. *Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma.* *J. Anim. Sci.* 71:753–756.
- Knapper, J. M. E., L. M. Morgan, and J. M. Fletcher. 1996. Nutrient-induced secretion and metabolic effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1. *Proc. Nutr. Soc.* 55:291–305.
- Kramer, J. K., V. Fellner, M. E. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. *Lipids* 32:1219–1228.
- Kumar, D., M. A. Foetschel, T. D. Pringle, and D. H. Keisler. 2004. Cholecystokinin mediates intake regulation of high fat diets in ruminants by acting on the reticulo-omasal sphincter. *J. Dairy Sci.* 87(Suppl. 1):309. (Abstr.)
- Litherland, N. B., S. Thire, A. D. Beaulieu, C. K. Reynolds, J. A. Benson, and J. K. Drackley. 2005. Dry matter intake is decreased

- more by abomasal infusion of unsaturated free fatty acids than by unsaturated triglycerides. *J. Dairy Sci.* 88:632–643.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute Inc., Cary, NC.
- Lundy, F. P., E. Block, W. C. Bridges, Jr., J. A. Bertrand, and T. C. Jenkins. 2004. Ruminant biohydrogenation in Holstein cows fed soybean fatty acids as amides or calcium salts. *J. Dairy Sci.* 87:1038–1046.
- Martin, P. A., A. Faulkner, and J. P. McCarthy. 1993. Effects of consumption of milk and milk constituents on plasma concentrations of gastric inhibitory polypeptide and metabolites in preruminant goat kids. *J. Endocrinol.* 138:167–173.
- Moran, T. H. 2004. Gut peptides in the control of food intake: 30 years of ideas. *Physiol. Behav.* 82:175–180.
- Morgan, L. M., B. A. Morris, and V. Marks. 1978. Radioimmunoassay of gastric inhibitory polypeptide. *Ann. Clin. Biochem.* 15:172–177.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Palmquist, D. L. 2006. Milk fat: Origin of fatty acids and influence of nutritional factors thereon. Pages 43–92 in *Advanced Dairy Chemistry*. Vol. 2: Lipids. P. F. Fox and P. L. H. McSweeney, ed. Springer, New York, NY.
- Reveneau, C., C. V. D. M. Ribeiro, M. L. Eastridge, N. R. St-Pierre, and J. L. Firkins. 2005. Processing whole cottonseed moderates fatty acid metabolism and improves performance by dairy cows. *J. Dairy Sci.* 88:4342–4355.
- Reynolds, C. K., G. B. Huntington, T. H. Elsasser, H. F. Tyrrell, and P. J. Reynolds. 1989. Net metabolism of hormones by portal-drained viscera and liver of lactating Holstein cows. *J. Dairy Sci.* 72:1459–1468.
- Robertson, M. D., K. G. Jackson, B. A. Fielding, L. M. Morgan, C. M. Williams, and K. N. Frayn. 2002. Acute ingestion of a meal rich in n-3 polyunsaturated fatty acids results in rapid gastric emptying in humans. *Am. J. Clin. Nutr.* 76:232–238.
- Samuelsson, B., K. Uvnäs-Moberg, R. C. Gorewit, and K. Svennersten-Sjaunja. 1996. Profiles of the hormones somatostatin, gastrin, CCK, prolactin, growth hormone and cortisol. I. In dairy cows that are milked and fed separately or milked and fed simultaneously. *Livest. Prod. Sci.* 46:49–56.
- SAS Institute. 2000. The SAS System for Windows. Release 8.1. SAS Institute, Inc., Cary, NC.
- Schauff, D. J., and J. H. Clark. 1992. Effects of feeding diets containing calcium salts of long-chain fatty acids to lactating dairy cows. *J. Dairy Sci.* 75:2990–3002.
- Shimizu, I., M. Hirota, C. Ohboshi, and K. Shima. 1987. Identification and localization of glucagon-like peptide-1 and its receptor in rat brain. *Endocrinology* 121:1076–1082.
- Sukhija, P. S., and D. L. Palmquist. 1990. Dissociation of calcium soaps of long-chain fatty acids in rumen fluid. *J. Dairy Sci.* 73:1784–1787.
- Tachibana, S., T. Onaga, H. Mineo, and S. Kato. 1995. Role of endogenous CCK in regulation of interdigestive pancreatic exocrine secretion in sheep (*Ovis aries*). *Comp. Biochem. Physiol. A Physiol.* 112:103–109.
- Thomsen, C., O. Rasmussen, T. Lousen, J. J. Holst, S. Fenselau, J. Schrezenmeir, and K. Hermansen. 1999. Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. *Am. J. Clin. Nutr.* 69:1135–1143.
- Turton, M. D., D. O'Shea, I. Gunn, S. A. Beak, C. M. Edwards, K. Meeran, S. J. Choi, G. M. Taylor, M. M. Heath, P. D. Lambert, J. P. Wilding, D. M. Smith, M. A. Ghatei, J. Herbert, and S. R. Bloom. 1996. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69–72.