Nutrient Requirements and Interactions

High Fat Diets Increase Plasma Cholecystokinin and Pancreatic Polypeptide, and Decrease Plasma Insulin and Feed Intake in Lactating Cows¹,²

BYUNG-RYUL CHOI AND DONALD L. PALMQUIST³

Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691-4096

ABSTRACT

High fat diets often decrease feed intake in dairy cows; however, mechanisms underlying fat-induced depression of feed intake are yet to be established. The postulate that high fat diets decrease feed intake by increasing concentrations of lipid metabolites or satiety hormones in blood was tested by using eight multiparous Holstein cows in a simultaneously replicated 4 x 4 Latin-square design. Treatments were control diet with 1) no fat added, 2) 30 g/kg calcium salts of long-chain fatty acids, 3) 60 g/kg calcium salts of long-chain fatty acids, and 4) 90 g/kg calcium salts of long-chain fatty acids. Cows were fed once daily a diet of concentrate, corn silage, alfalfa haylage and alfalfa hay (50:25:14:11 on a dry matter basis). Dry matter and energy intakes were decreased by inclusion of calcium salts of long-chain fatty acids >30 g/kg of total diet dry matter (P = 0.0001). Plasma nonesterified fatty acids and triglyceride concentrations were increased linearly by feeding increasing amounts of fat (P < 0.003 and P = 0.0001, respectively), whereas plasma β-hydroxybutyrate and glucose concentrations were not influenced by supplemental fat. Fat supplementation increased postfeeding plasma cholecystokinin concentrations and linearly increased plasma pancreatic polypeptide concentrations. Highest concentrations of plasma cholecystokinin (P < 0.001) and pancreatic polypeptide (P < 0.05) were observed in cows fed the 90 g/kg fat supplement. Plasma insulin was lowered linearly by feeding fat (P = 0.0001). Increased concentrations of cholecystokinin and pancreatic polypeptide were associated with decreased intakes of feed and energy, whereas insulin may not be involved in the control of feed intake in cows fed fat. J. Nutr. 126: 2913–2919, 1996.

INDEXING KEY WORDS:

- dietary fat
- feed intake
- cholecystokinin
- pancreatic polypeptide
- dairy cows

Maximum milk production requires maximum feed and energy intake by high producing dairy cows. The addition of fat to lactation rations increases energy density of the diet and milk production. Despite potential improvements in energy intake and milk yield, additional fat often causes a marked reduction in feed and energy intake when fed to cows in amounts exceeding 50–60 g/kg of diet dry matter (Schauff and Clark 1992). Gastrointestinal infusion of long-chain fatty acids also suppressed dry matter intake in cows (Christensen et al. 1994).

Although mechanisms regulating fat-induced depression of dry matter intake have not been investigated fully in cows, several factors are known. Fat may inhibit fiber digestion in the rumen (Palmquist and Jenkins 1980), which may increase rumen fill by the undigested feed residues, thereby decreasing dry matter intake (Conrad et al. 1964). However, the fiber digestion problem can be eliminated by feeding ruminally inert fats (Ohajuruka et al. 1991). It was postulated that when cows consume large amounts of fat which cannot be metabolized, feedback satiety signals may be generated to prevent further influx of fuels (Palmquist 1994). Because satiety signals are integrated and processed in the brain, signals informing the brain of peripheral satiety status elicited by fat should be present. Lipid metabolites in the systemic circulation possibly serve this role. Reducing equivalents generated by fatty acid oxidation are reportedly involved in fat-induced depression of feed intake in rats (Sharrer and Langhans 1986). The most extensively studied, however, are satiety hormones. Intravenous injection of cholecystokinin

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³ To whom correspondence should be addressed.

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2913
(CCK)\textsuperscript{4} in sheep (Grovum 1981), intravenous injection of insulin in cows (Faverdin 1986), and intraperitoneal injection of pancreatic polypeptide (PP) in mice (McLaughlin and Baile 1981) reduced intake.

Because fat is a potent releaser of CCK (Liddle et al. 1985), insulin (Opara et al. 1994) and PP (Taylor 1989), these hormones are potential regulators of feed intake of cows fed fat. No information, however, is available concerning the responses of CCK and PP to high fat diets in ruminants. The objective was to establish whether high fat diets fed to lactating cows elicit increases in CCK and PP, and to determine whether these may be related to feed intake and concentrations of plasma metabolites. The experimental design was modeled on that reported by Schaff and Clark (1992), who demonstrated a clear response of feed and energy intakes to increasing amounts of fat in the diets of lactating cows.

**MATERIALS AND METHODS**

**Experimental design, diets and management of cows.** Animal care and management subscribed to principles approved by The Ohio State University Animal Care Committee. Eight multiparous Holstein dairy cows averaging 115 d in milk were used in a simultaneously replicated 4 × 4 Latin-square design with 14-d feeding periods. Each period consisted of 7 d for cows to adapt to diets and 7 d for sample collection. Treatments were four diets to which calcium salts of long-chain fatty acids [Megalac\textsuperscript{®}, Church and Dwight, Princeton, NJ] were added at 0, 30, 60 and 90 g/kg of total dry matter. Ingredients and chemical composition of the diets are listed in Table 1. All diets were balanced with soybean meal and blood meal to contain approximately equal weights of concentrates in diets 2–4 on a dry matter basis. Calcium salts of long-chain fatty acids (CSFA) replaced an approximately equal weight of concentrates in diets 2–4 on a dry matter basis. The forage (corn silage, alfalfa hay and alfalfa haylage) was 50% of total dry matter for all treatments. Animals were fed the diets once daily at 0730 h and milked twice daily at 0200 and 1300 h.

**Dry matter intake and milk production.** Dry matter intake was measured and recorded daily. Net energy for lactation (NE\textsubscript{L}) intake was estimated by difference between NE\textsubscript{L} offered and that inorts. Net energy values of the diets and orts were estimated from their contents of acid detergent fiber (ADF), using an equation that was constructed from ADF and energy values of feeds (NRC 1989). Feed ingredients and orts were collected three times during the last 7 d of each period in propor-

![Table 1](https://academic.oup.com/jn/article-abstract/126/11/2913/4724726)

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<th>Ingredient</th>
<th>0</th>
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<td>Alfalfa hay</td>
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</tr>
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<td>7.4</td>
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<td>CSFA\textsuperscript{2}</td>
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<td>4.0</td>
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<td>7.03</td>
<td>7.41</td>
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</table>

1 Formulated to meet or exceed nutrient requirements (NRC 1989).
2 Calcium salts of long-chain fatty acids marketed as Megalac\textsuperscript{®} by Church & Dwight, Princeton, NJ.
3 Contained (per kg mixture). 3.44 g of retinyl acetate, 0.025 g of cholecalciferol, and 15 g of DL-α-tocopherol acetate.

The amount of feed offered and the amount of orts. Composited feed and orts samples were lyophilized, ground in a Wiley mill through a 1-mm screen (Arthur H. Thomas, Philadelphia, PA), and analyzed for neutral detergent fiber, ADF and total fatty acids, as described by Ohajuruka et al. (1991). Fresh samples of feed and orts were analyzed for nitrogen (Ohajuruka et al. 1991). Milk yield was recorded daily, and milk samples were collected at individual milkings twice in each period. Contents of milk fat and protein were determined by Ohio Dairy Herd Improvement (Powell, OH) using infrared procedures.

**Blood sampling and analysis.** Blood samples were drawn from the coccygeal vein into 10-mL heparinized tubes [5000 USP units of heparin/L whole blood] at 0, 1, 3 and 6 h after feeding. Plasma samples were harvested after centrifugation at 3000 × g for 15 min at 4°C and stored at −27°C until analyzed. Plasma samples for CCK and PP assay were stored in tubes containing 250 KIU aprotinin/L and kept frozen at −27°C. Plasma non-

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\textsuperscript{4} Abbreviations used: ADF, acid detergent fiber; BHBA, β-hydroxybutyrate; CCK, cholecystokinin; CSFA, calcium salts of long-chain fatty acids; NEFA, nonesterified fatty acids; NE\textsubscript{L}, net energy for lactation; PP, pancreatic polypeptide.
estherified fatty acids (NEFA) [Wako NEFA C Kit, Wako Chemical, Osaka, Japan], triglyceride (Sigma procedure #336, St. Louis, MO) and glucose (Sigma procedure #510), were determined by micromethods and analyzed by using an ELISA plate reader. Plasma for β-hydroxybutyrate (BHBA) determination was immediately deproteinized and stored at –27°C until assayed (Williamson and Mellanby 1972). Insulin was assayed by using a RIA kit [Coat-a-Count®, Diagnostic Products, Los Angeles, CA]. Inter- and intraassay coefficients of variation for insulin assay were 13 and 7%, respectively. Assay for CCK was by Dr. P. L. Rayford [Medical Sciences, University of Arkansas] using a double antibody RIA technique with UT122, the first antibody specific to the N-terminal peptide of CCK (Rayford and McKay 1986). Inter- and intraassay CV were 17 and 6%, respectively. Assay for PP was by Dr. T. M. O’Dorisio [Medical School, The Ohio State University] using a double antibody RIA procedure with human PP antiserum as the first antibody (Zipf et al. 1983). Inter- and intraassay CV were 15 and 10%, respectively.

Statistical analysis. All daily measures of dry matter, energy and nutrient intakes, and milk yields were reduced to means by period before analysis by ANOVA for a Latin-square design with the GLM procedure of SAS® [SAS Institute, Cary, NC]; results are reported as least-squares means (LSmeans). Squares were analyzed as replicates. The statistical model used for analysis of intake and production measures was as follows:  \[ Y_{ijkl} = \mu + P_i + C_j + T_k + E_{ijkl} \]  where \( \mu \) = overall mean; \( P_i \) = average effect of the \( i \)th period, \( i = 1-4 \); \( C_j \) = average effects of the \( j \)th cow, \( j = 1-8 \); \( T_k \) = average effect of the \( k \)th treatment, and \( E_{ijkl} \) = the unexplained residual error. Orthogonal comparisons for treatments were linear and quadratic effects.

The data for plasma measures taken over time also were analyzed in the above-described statistical model by using the repeated measures options of GLM (SAS). Because time by diet interactions were not observed among blood measures, time effects were evaluated using pooled treatment means by multiple contrast. Additional analyses of treatment responses at individual sampling times were made by single contrast. Differences between treatment means were considered to be significant at \( P = 0.05 \). Probability values between 0.05 and 0.15 were considered to indicate a trend toward a significant effect.

RESULTS

Dry matter and nutrient intakes, and milk production. Dry matter and NE\(_i\) intakes decreased linearly \( (P = 0.0001) \) as increasing amounts of CSFA were fed to cows [Table 2]. Additional fat also linearly decreased crude protein, ADF and neutral detergent fiber intakes \( (P = 0.0001) \). Yields of actual and energy-corrected milk were increased quadratically with maximum at 6% fat \( (P < 0.005 \) and \( P = 0.001 \), respectively). Milk fat content was increased linearly by feeding increasing amounts of fat \( (P < 0.05) \). Feeding fat did not alter milk protein content but linearly decreased milk protein production \( (P = 0.0005) \).

Plasma metabolites. No time by diet interactions were observed among plasma metabolites. Feeding fat linearly increased plasma NEFA [Fig. 1] and triglyceride [Fig. 2] concentrations at all sampling times \( (P < 0.003 \) and \( P = 0.0001 \), respectively). Prefeeding values also were increased linearly by feeding fat for NEFA \( (P < 0.04) \) and triglyceride \( (P < 0.002) \), respectively. Posterfeeding plasma NEFA concentrations decreased from prefeeding values \( (P = 0.002, 0.0004 \) and 0.0009 at 1, 3 and 6 h postfeeding, respectively). Plasma triglyceride concentrations were lower at 1 and 6 h postfeeding compared with prefeeding concentrations \( (P < 0.03 \) and \( P = 0.0009 \), respectively). Plasma BHBA concentrations [Fig. 3] were not influenced by the amount of fat fed but gradually increased after feeding \( (P = 0.0001) \). Glucose concentrations [Fig. 4] were not affected by the diets after feeding, whereas prefeeding values were decreased by feeding fat \( (P < 0.03) \). Plasma glucose concentrations decreased gradually as sampling time proceeded \( (P = 0.0001) \). Changes in plasma concentrations of glucose, NEFA and triglyceride were responsive to changes in plasma insulin concentrations after feeding.

Plasma satiety hormones. No time by diet interactions were observed among plasma hormones. Plasma CCK concentrations are shown in Figure 5. Although a time by diet interaction was not significant, feeding the control diet decreased plasma CCK concentration at 3 and 6 h postfeeding from the prefeeding value \( (P = 0.0007 \) and \( P < 0.08 \), respectively). Fat-supplemented diets did not change postfeeding plasma CCK concentrations. However, increasing fat in the diets tended to cause higher plasma CCK concentrations relative to the control \( (P = 0.13) \). Cholecystokinin concentrations were numerically but not significantly higher in plasma of cows fed the high fat diets at 0 and 1 h after feeding, compared with the control; at 3 h postfeeding, concentrations were linearly higher \( (P = 0.02) \) and at 6 h also tended to be increased \( (P < 0.1) \) by feeding increasing amounts of fat. Continuously highest CCK concentrations were observed in cows fed the 9% CSFA diet.

Plasma PP concentrations [Fig. 6] were elevated at 1 and 3 h postfeeding compared with the prefeeding concentration \( (P < 0.002 \) and \( P < 0.007 \), respectively), and apparently were increased by feeding fat \( (P = 0.1) \) with a peak at 1 h postfeeding. A linear effect of fat on PP concentration was significant at 3 h \( (P = 0.03) \) and 6 h postfeeding \( (P < 0.05) \). Cows fed the 90 g/kg fat diet had the continuously highest postprandial concentrations of PP in plasma.

Plasma insulin concentration [Fig. 7] increased gradually after feeding \( (P = 0.0001) \). Although prefeeding
concentrations were similar for all diets, postprandial increases in plasma insulin concentrations were decreased markedly in cows fed fat [P < 0.002]. The effect of dietary fat on insulin became apparent from 1 h postfeeding.

DISCUSSION

Feeding fat usually increases plasma NEFA and triglyceride concentrations in cows (Grummer and Carroll 1991, Palmquist and Conrad 1978). The gradual decrease in NEFA concentrations after feeding were consistent with postprandial increases in plasma insulin concentrations. Plasma BHBA concentration was not changed with increasing dietary fat and seemingly reflected the rate of butyrate production from rumen fermentation rather than fatty acid oxidation. Plasma glucose was not altered by feeding fat, which is consistent with other studies (Grummer and Carroll 1991, Palmquist and Conrad 1978). Changes in plasma metabolites were within physiologically normal ranges. Changes in plasma CCK concentrations were not influenced by feeding, except in the control group where CCK markedly declined from prefeeding concentrations.

**FIGURE 1** Plasma nonesterified fatty acid (NEFA) concentrations from 1 h prefeeding to 6 h in cows fed 0–90 g/kg supplemental fat postfeeding. Legends are percent calcium salts of long-chain fatty acids [CSFA] added to diets. Values are means ± standard errors, n = 8. Symbols *, **, and *** indicate diet effects at P < 0.05, 0.001, and P = 0.0001, respectively. Overall diet and time effects were significant [P < 0.003 and P = 0.0001, respectively].

**FIGURE 2** Plasma triglyceride concentrations from 1 h prefeeding to 6 h postfeeding in cows fed 0–90 g/kg supplemental fat. Legends are percent calcium salts of long-chain fatty acids [CSFA] added to diets. Values are means ± standard errors, n = 8. Symbol * indicates a diet effect at P < 0.05. Overall diet and time effects were significant (P = 0.0001).
FIGURE 3 Plasma β-hydroxybutyrate (BHBA) concentrations from 1 h prefeeding to 6 h postfeeding in cows fed 0–90 g/kg supplemental fat. Legends are percent calcium salts of long-chain fatty acids (CSFA) added to diets. Values are means ± standard errors, n = 8. The time effect was significant (P = 0.0001). The overall diet effect was not significant.

FIGURE 4 Plasma glucose concentrations from 1 h prefeeding to 6 h postfeeding in cows fed 0–90 g/kg supplemental fat. Legends are percent calcium salts of long-chain fatty acids (CSFA) added to diets. Values are means ± standard errors, n = 8. Symbol * indicates a diet effect at P < 0.05. The overall time effect was significant (P = 0.0001) but the overall diet effect was not significant.

FIGURE 5 Plasma cholecystokinin (CCK) concentrations from 1 h prefeeding to 6 h postfeeding in cows fed 0–90 g/kg supplemental fat. Legends are percent calcium salts of long-chain fatty acids (CSFA) added to diets. Values are means ± standard errors, n = 8. Symbol * indicates a diet effect at P < 0.05. Overall diet and time effects were at P < 0.13 and P < 0.02, respectively.

FIGURE 6 Plasma pancreatic polypeptide (PP) concentrations from 1 h prefeeding to 6 h postfeeding in cows fed 0–90 g/kg supplemental fat. Legends are percent calcium salts of long-chain fatty acids (CSFA) added to diets. Values are means ± standard errors, n = 8. Symbol * indicates a diet effect at P < 0.05. Overall diet and time effects were at P < 0.1 and P = 0.0003, respectively.
came significant from 3 h postfeeding with a linear increase in response to increasing amounts of dietary fat. Like CCK, PP secretion is biphasic with an early peak followed by a prolonged plateau (Taylor 1989). CCK is a known PP releaser (Taylor 1989), and CCK and PP were correlated ($r = 0.34$, $P < 0.05$; Table 3). Our data suggest that PP secretion may be regulated by CCK in cows.

The lower plasma insulin concentrations observed when feeding dietary fat were consistent with other reports (Khorasani et al. 1992, Palmquist and Moser 1981). Increasing unsaturation of long-chain fatty acids linearly increased in vitro insulin release in nonruminants (Opara et al. 1994). Possibly, fat supplements rich in saturated fatty acids, such as typically absorbed by ruminants, may have a negative effect on plasma insulin concentration in cows. Decreased dry matter intake also may cause lowered insulin release in cows fed fat. Propionate, largely produced from ruminal fermenta-

![FIGURE 7](https://academic.oup.com/jn/article-abstract/126/11/2913/4724726)

**FIGURE 7** Plasma insulin concentrations from 1 h prefeeding to 6 h postfeeding in cows fed 0–90 g/kg supplemental fat. Legends are percent calcium salts of long-chain fatty acids (CSFA) added to diets. Values are means ± standard errors, $n = 8$. Symbols * and ** indicate diet effects at $P < 0.05$ and $P < 0.001$, respectively. Overall diet and time effects were significant ($P < 0.0002$ and $P = 0.0001$, respectively).

<table>
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<tr>
<th>Measure</th>
<th>NEFA$^2$</th>
<th>Glucose</th>
<th>Insulin</th>
<th>CCK</th>
<th>PP</th>
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<tbody>
<tr>
<td>Dry matter intake</td>
<td>−0.322$^a$</td>
<td>−0.287$^b$</td>
<td>0.178</td>
<td>−0.147</td>
<td>−0.097</td>
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<tr>
<td>NEFA</td>
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<td>Insulin</td>
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<td>CCK</td>
<td>0.338$^a$</td>
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1 Included prefeeding measures. $^aP < 0.001$, $^bP < 0.05$.
2 NEFA, non-esterified fatty acids; CCK, cholecystokinin; PP, pancreatic polypeptide.
tration. Hence, an inhibitory effect of intravenously administered exogenous insulin on feed intake might be mediated via pharmacological rather than physiological mechanisms (Grovum 1995).

In conclusion, feeding increasing amounts of dietary fat linearly decreased feed and energy intakes and linearly increased plasma CCK and PP concentrations in lactating cows. Although direct evidence remains to be gained, we suggest that decreased feed intake in cows fed high fat diets is mediated by increased plasma CCK and PP concentrations. Insulin may not be a factor mediating fat-induced depression of feed intake in cows. The amount of fat added to lactation rations should be determined from milk production potential, or actual milk fat yield of cows in order to maintain high dry matter intake.

ACKNOWLEDGMENTS

The authors thank Phillip L. Rayford [University of Arkansas, Medical Sciences] for CCK assay, and Thomas M. O’Dorisio [The Ohio State University, Medical School] for PP assay. We appreciate the advice of Bert Bishop [OARDC] on data analyses. Also, appreciation is extended to the dairy barn crew at OARDC for excellent care and management of cows.

LITERATURE CITED


