LACTATIONAL, METABOLIC, AND PHYSIOLOGICAL EFFECTS OF DIETARY FATS AND ISOACIDS ON EARLY LACTATING FIRST-CALF HOLSTEIN HEIFERS

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(ABSTRACT)

Forty four first-calf heifers were randomly selected to determine lactational and metabolic responses to high fat diets and isoacids. All heifers were allowed ad libitum consumption of a control diet for the first 2 weeks of lactation and then offered experimental diets for the next 4 weeks. Each 6 cows of twenty four were randomly assigned to 1) a control diet (C) with 35.2% corn silage, 14.4% alfalfa haylage and 50.4% concentrate (dry matter basis), 2) C with 2% calcium stearate (S) substituted for corn grain, 3) C with 2% tallow (T) for corn grain, and 4) C with 10% whole cottonseed (W) for corn grain, cottonseed meal and cottonseed hulls. The remaining 20 heifers were randomly assigned to diets C, S, T, and W, each with 4g/kg isoacids added (CI, SI, TI, and WI). Fat supplementation or isoacid addition did not affect milk production. Addition of isoacid increased milk fat percentage, 4% fat-corrected milk, milk fat production

(kg/day) and dry matter intake. Differences due to isoacid were greatest when added to W. Increased milk lactose percentage and weight gain were evident in animal receiving WI compared to W ration. Fat supplementation depressed percentages of milk fat, milk lactose and milk solids-not-fat. Milk protein percentage and somatic cell count were not affected by treatments. Plasma glucose,and glucose and epinephrine challenge parameters were not affected by diet. Peak plasma non-esterified fatty acid response to epinephrine injection, detected at 10 to 12 minites, was similar for C, S, T, and W. Concentrations of individual volatile fatty acids (VFA) and total VFA in rumen fluid were increased by fat supplements. Isoacid addition increased the amounts of isobutyrate and isovalerate; however, acetate and total VFA concentrations were decreased compared to CI when isoacids were added to high fat diets. The ratio of acetate to propionate was similar for all diets. Digestibilities of dry matter, crude protein, and acid detergent fiber were not influenced by diet. The efficiency of energy utilization was highest for control diet. In conclusion, lactational, metabolic, and physiological responses to S, CI, and WI were favorable. Responses to W were lowest.

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INTRODUCTION

Fat supplementation has been recommended to raise energy concentration of dairy cattle diets in early lactation when energy balance is negative. Fat has been used successfully to increase the ratio of forage to concentrate by replacing dietary grains (94, 95). Polyunsaturated oils and tallow, however, have a tendency to depress milk fat content and ruminal digestive function. Long-chain fatty acids apparently disturb ruminal microbial metabolism and growth (34, 75, 94, 122, 127).

A potential for increased milk fat yield and normal ruminal digestion has been demonstrated by protected dietary lipids (50). The results of many studies (8, 10, 11, 44, 75, 99, 106, 113, 126, 127, 128) suggested that dairy cows responded positively to formaldehyde-treated casein-oils or protected tallows by consistently producing more milk and milk fat.

In recent years, whole cottonseed has been of interest because it is in a naturally protected form. A few studies (3, 33) have reported that whole cottonseed feeding resulted in the high production of milk fat and fat-corrected milk. However, DePeters et al. (33) has suggested that whole

cottonseed tended to depress milk protein content due to decreased milk casein nitrogen.

Calcium-bound fatty acid feeding has been widely studied (51, 57, 58, 64, 96). Insoluble calcium soaps bypassed rumen fermentation and were absorbed in the lower digestive tract, as in whole cottonseed (57, 58, 96). As a result, dairy cows maintained favorable ruminal function and produced more milk fat (53, 58, 64).

Isoacid addition to the dairy ration increased protein microbial growth and fiber digestion resulting in increased milk yield (7, 30, 97). Isoacid addition to diets containing low levels of protein led to high persistency and production of milk (38). Increased milk yields may be attributed to higher dry matter digestion and greater microbial protein production in the rumen (30). Felix et al. (37) has reported increased growth rate of dairy heifers fed isoacid supplemented diets. However, no information is available on interactions or simultaneous feeding of supplemental isoacids and dietary fat.

Objectives of the present study were 1) to evaluate lactational, metabolic, and physiological effects of three sources of dietary fat (tallow, calcium soap, and whole cottonseed) fed to dairy cattle during the early part of their first lactation and 2) to investigate the possible interactions between isoacid and fat supplementation.

REVIEW OF LITERATURE

BENEFITS OF FEEDING FAT

In the past 35 years, the average yearly milk production per cow in the U.S. has increased more than 120% (26). This successful performance has been attributed to genetic improvement of dairy cows, increased supplies of forage and grain, and development of animal nutrition (54). Many studies by animal nutritionists have focused on the optimal quantities of feedstuffs (26) for higher and more economical milk yield.

In early lactation, nutrient requirements of lactating cows are greater than their ability to consume dry matter. As a result, energy balance is negative. High-producing cows have difficulty meeting energy requirements and maintaining high levels of milk production once peak dry matter intake is attained. Cows have a limited digestive tract capacity (91, 100), which limits energy intake for (91). To maximize feed intake, several factors need to be considered: ratio of forage to concentrate, moisture level of feedstuffs, feed processing method, energy to protein ratio, and supplements containing buffers, proteins, or fat (26).

Dairy rations normally contain 3 to 4% fat (90, 95). An increase in the fat content of the diet enables greater consumption of energy and higher efficiency of energy utilization at a constant dry matter intake. Fat contains over two times as much energy as protein or carbohydrate. Fat can replace starch in the diet (50, 94, 95), allowing an increased ratio of forage to concentrate and avoidance of milk fat depression caused by excess starch feeding (95). However, unprotected fat also can cause negative effects such as decreased fiber digestion and a low acetate to propionate ratio (90). In contrast, protected fat has resulted in favorable responses. When fed protected oil, cows produced milk and meat containing highly unsaturated fat (8).

CLASSIFICATION OF FAT SUPPLEMENTS

Supplementary fats or oils can be fed in an unprotected form or in a protected form encapsulated in a casein-formaldehyde or formalin coating. From the late 60's until the late 70's, many studies had been conducted on diets containing protected or unprotected fats or oils; i.e., safflower oil, soybean oil, cottonseed oil, coconut oil and codliver oil as polyunsaturated oils, and whole soybean seed, full-fat soyflour, sunflower seed, tallow, and mixtures of

two supplements (i.e., sunflower-soybean, or blended animal-vegetable fat).

In recent years, whole cottonseed has been a popular source of added fat. Furthermore, increasing interest has been shown in commercial products such as ammonium or calcium salts of isoacids or volatile fatty acids, and calcium stearate. It is expected that fat supplements will continue to be improved in the future.

DIETARY OILS

In 1968, Varman et al. (122) reported that feeding daily 250 ml of safflower oil depressed percent milk fat, percent ruminal acetate, and plasma triglyceride. It elevated plasma non-esterified fatty acids, plasma cholesterol, and proportions of all the volatile fatty acids except acetate in the rumen. In a subsequent study(102), the same amount of safflower oil was infused abomasally over 4 hours daily for 3 weeks. Results of this study suggested that abomasal infusion of safflower oil increased percent milk fat, blood ketones, non-esterified fatty acid, and cholesterol, and percent linoleic acid (C18:2) in milk fat whereas it reduced percent myristic, palmitic, stearic, and oleic acids in milk fat.

Plowman et al. (99) and Wrenn et al. (126) suggested that safflower oil-casein protected with formaldehyde resulted in increased percent milk fat, milk linoleic acid (C18:2) with reduced short chain fatty acids (C8:0-16:0) due to the inhibited mammary de novo short chain fatty acid synthesis from long-chain fatty acids (95). The latter worker further observed that although protected safflower oil elevated blood cholesterol, milk and meat cholesterol levels were unchanged, indicating no detrimental effect from feeding protected safflower oil. In general, dairy cows have responded more favorably to the protected safflower oil.

Astrup et al. (8) fed each 400 g of protected coconut oil, protected hydrogenated soybean oil, and protected soybean oil to 6 dairy cows for 4 weeks. In this experiment, the workers observed increased percent milk fat and milk dry matter, serum cholesterol, and serum total lipid in both of protected coconut and soybean oil treatments, and decreased percent milk fat and milk dry matter in protected hydrogenated soybean oil treatment. These results were similar to the report of Goering et al (44). The workers concluded that feeding protected soybean oils would be more beneficial than feeding unprotected one.

In contrast, by feeding unprotected soybean oil, percent milk fat was depressed (72) or unchanged (44, 71). Percent

milk stearic (C18:0) and oleic acid (C18:1) increased as percentages of short chain fatty acids and linoleic acid (C18:2) in milk fat were reduced (44,72). Dry matter intake was unchanged (44) or slightly decreased (71). Digestible energy intake, fat-corrected milk, percent solids-not-fat, plasma triglyceride and cholesterol were not changed (44). These data showed that dairy cows responded positively to the unprotected soybean oil. According to the data of Mattos et al. (75), 3.6 kg/day unprotected or protected full-fat soyflour was successfully fed to dairy cows with increased milk yield and fat contents.

Storry et al. (113) found that when free or protected codliver oil was fed to dairy cows for 22 days, yield of long chain fatty acids (C:20-22) in milk fat was significantly elevated. These workers further showed that cows were less responsive to free codliver oil, resulting in the depressed acetate to propionate ratio in the rumen.

Yang et al. (128) fed 15% sunflower seeds treated with formalin to 10 dairy cows in the second month of lactation and normal milk production was maintained. Decreased percentages and yields of myristic and palmitic acids in milk fat were compensated for by increased stearic and linoleic acids.

Barbano et al (11) showed positive effects of protected

sunflower-soybean supplement for dairy cows. Production of milk phospholipid and fat was increased while milk yield was unaltered.

DIETARY TALLOW

In recent years, several studies (67, 68, 73, 108, 110) have been conducted to determine the effect of protected tallow supplement on lactating cows. The researchers have reported that protected tallow can be successfully fed to dairy cows.

According to the studies, milk production was normally maintained, and percent milk fat and fat-corrected milk were significantly increased (68, 73, 108, 110). Percent milk protein (73, 108) and lactose (73) were negatively influenced. Efficiency of utilization of metabolizable energy for lactation was increased 8.0 - 13.6% in the diet containing 25% of metabolizable energy as protected tallow (68). Plasma glucose was not affected (68, 110) and plasma cholesterol (68, 110), and triglyceride (110) were elevated. Digestibilities of crude protein and crude fiber were unaltered while digestibility of lipids was influenced positively (73, 108). The acetate to propionate ratio was significantly elevated (90).

Tallow is composed of 60% saturated fatty acids and approximately 40% of unsaturated fatty acids, and its melting point is 42°C (76).

DePeters et al. (34) fed 0, 3.5, and 7% animal fat to 12 lactating Holstein cows for 3 weeks. For 7% fat diet, percentages of all the milk compositions and percent casein nitrogen in milk protein were influenced negatively. Digestibilities tended to decrease with increasing dietary fat.

According to the results of four studies (87, 94, 112, 127) when dairy cows were given 5 to 6% dietary tallow, milk production was increased (127) or unchanged (87, 94, 112). Percent palmitoleic (127), stearic (112), and oleic acids (112, 127) were elevated while carbon chain 6 to 16 fatty acids were reduced (112). Storry et al. (112) suggested that a 2% tallow diet significantly changed the relative proportions of fatty acids in milk fat. Percent milk protein was not reduced (87, 94, 127). Body weight responded positively (127) or insignificantly (94). No abnormal digestibilities and proportions of ruminal volatile fatty acids were detected when fed up to 6% dietary tallow (87, 94). It has been shown that tallow has a tendency to decrease fiber digestibility (58, 91, 95). Palmquist et al. (94) confirmed that digestibilities of all the components

were significantly increased by the addition of calcium. Plasma lipids and plasma cholesterol were increased (127). Feed intake was not influenced (127) or was slightly decreased (94). Heinrichs et al. (49) has suggested that cows had a tendency to consume a small amount of tallow diet frequently, and that the total feed intake was usually not different from unsupplemented diets.

Macleod et al. (71) used hydrogenated tallow to determine the effect of the saturated fat on milk yield and composition. This study showed that hydrogenated tallow did not influence milk yield and composition, or proportions of ruminal volatile fatty acids. Only slight decreases in feed intake and concentration of blood glucose were detected. Similarly, in another experiment by Macleod et al. (72), proportions of milk fatty acids were not affected by the hydrogenated tallow while plasma lipid fractions were increased.

CALCIUM SOAPS

The unprotected fat often inhibits fiber digestion in the rumen, resulting in the low acetate to propionate ratio followed by milk fat depression (58). To remove this inhibitory effect by fat, two techniques have been studied.

The first technique was to add calcium to the dietary fat to form insoluble calcium soaps in the rumen (57, 96). It has been presumed that the insoluble soaps formed in the rumen should escape the rumen fermentation and dissociate postruminally (58) into calcium cations and free fatty acids, which may be utilized directly for milk synthesis.

Jenkins et al. (57) conducted an in vitro test to evaluate how ruminal formation of insoluble soaps and fiber digestion would be influenced by added fat and calcium. Approximately 60% of total fatty acids formed insoluble soaps in the rumen. Added calcium chloride influenced formation of soaps and fiber digestion more positively due to its higher solubility than relatively insoluble dicalcium phosphate. However, depressed fiber digestion was observed due to the long-chain fatty acids produced in the rumen.

In the subsequent in vivo study of Palmquist et al. (96), it has been suggested that formation of soaps was strongly dependent on the amount of ruminal fat and was not affected by the added calcium although the improved digestion by calcium supplement was still observed. The researchers have concluded that the favorable influence of the added calcium and fat was not attributed to the increased formation of calcium soaps in the rumen, noting that excessive additional calcium may adversely affect by reforming insoluble soaps in

the lower digestive tract and being excreted in the feces (58). However, the optimal amount of calcium required in fat diet remains unclear. This first technique should be further studied.

The second technique is to add calcium and fat as preformed calcium soaps. Jenkins et al. (58) found that feeding preformed tallow calcium soap alleviated reduced digestibility of crude fiber caused by 4.5% dietary tallow fatty acid, and conversely, reduced digestibilities of fatty acids and energy by 6% and 3%, respectively.

In another study (64), calcium-treated soaps were higher in digestibilities, serum lipids and cholesterol than animal fat. Herbein et al. (51) tested the effects of preformed calcium stearate and tallow on 44 early lactating cows. The calcium stearate diet was highest in fat-corrected milk, acetate to propionate ratio, and growth hormone to insulin ratio. Plasma non-esterified fatty acids, calcium and magnesium were not influenced by calcium stearate diet.

The workers (51, 58) concluded that preformed calcium soaps were effective and beneficial when fed to dairy cows to maximally utilize dietary fats.

WHOLE COTTONSEED

Whole cottonseed (WCS) contains fat, protein, and highly digestible fiber (3, 18, 28, 33, 48, 70). It has been recommended for feeding in the hot season when lactating cows may suffer from heat stress, resulting in depressed dry matter intake and milk and milk fat production (28, 29). Although WCS is a widely used dietary ingredient, it is still unclear how WCS affects milk composition of lactating cows under all conditions (33).

Anderson et al. (3) reported that WCS feeding increased milk production; however, other studies (29, 33, 48) have not observed any difference in milk production. DePeters et al. (33) suggested that percent milk fat, milk total solid, and fat-corrected milk increased with WCS feeding, whereas other studies (3, 29, 48) showed no difference in percent milk fat and fat corrected milk. DePeters et al. (33) further suggested that dietary WCS decreased percent milk protein due to depressed milk casein, and it also decreased percent milk solids-not-fat. In contrast, other studies (3, 29, 48) have shown no change in percent milk protein. WCS decreased (29, 48) or had no effect on dry matter intake (3, 33). Also body weight gain was not affected (33).

In an experiment where 0, 15, and 30% WCS were fed,

Coppock et al. (29) observed increased blood calcium, decreased blood glucose, and increased digestibility of crude protein, apparently due to high crude protein in the diet. Hawkins et al. (48) reported that diets containing 18.5% WCS markedly increased plasma total lipids, cholesterol, and gossypol, primarily due to high lipid intake from the diet. He further suggested that WCS feeding did not change the duration of the lactation period; although, some fat supplemented diets have shown a shortened lactation period (91). Anderson et al. (3) observed no effect of WCS (1.9 kg per cow per day) on rumen volatile fatty acids, except a slight increase in the acetate to propionate ratio.

Some cows have responded favorably to WCS, but negative effects have been suggested. Lindsey et al. (70) and Hawkins et al. (48) warned that gossypol toxicity may result from feeding excessive WCS or cottonseed meal, although no actual cases of toxicity have been reported. In practice, WCS feeding costs may be higher relative to other feedstuffs. To be accepted economically, WCS feeding must show a significant increase in milk production, milk fat, or fat corrected milk (3). Accordingly, processes like pelleting prior to feeding may be considered (28).

Further study is required before lactational and metabolic responses to WCS feeding are completely understood.

AMMONIUM OR CALCIUM SALTS OF VOLATILE FATTY ACIDS

Feeding calcium or ammonium salts of volatile fatty acids has been known to increase milk production significantly (7, 25, 30, 37, 38, 65, 66, 88, 103, 104) and persistency of milk yield (7, 38, 65). The cationic neutralized volatile fatty acids are composed of isobutyric, isovaleric, valeric, and 2-methylbutyric acids (32). As shown in Figure 1, these isoacids are essential for growth of cellulolytic anaerobic bacteria and synthesis of microbial protein (2, 21, 32). The carboxylation reactions of branched-chain fatty acids produce branched-chain amino acids which are building blocks for microbial protein synthesis (21). Valeric acid produced by deamination of proline in vitro (2) improved cellulose digestion with branched-chain fatty acids (7, 30, 38).

Improved cellulose digestion and microbial protein synthesis may lead to higher milk production (7, 30, 38). Lower-producing cows have responded more positively to isoacids (7). Rogers (103) and Newman et al. (88) have reported higher response of milk yield for early lactating cows and for heifers fed ammonium salt or calcium salt of volatile fatty acids. In contrast, another study of Rogers et al. (104) has suggested that older cows showed more response.

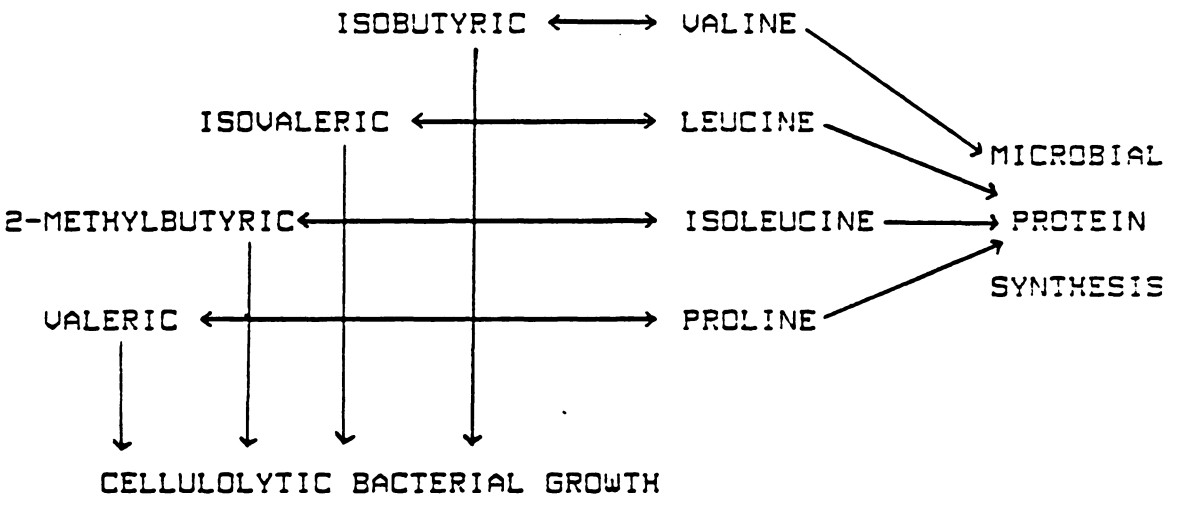

Figure 1. Correlationships between volatile fatty acids and amino acids for cellulolytic bacterial growth and microbial protein synthesis

to calcium salt of volatile fatty acids.

Percent milk fat was not changed (7, 88, 103), and effect of carryover milk yield was not significant after calcium salt of volatile fatty acids feeding was discontinued (25, 104). Body weight was slightly reduced for lactating cows (38) and was increased for younger cows when fed isoacids (37, 65). In a digestion trial, Felix et al. (37) reported that nitrogen utilization was increased by adding isoacid while digestibilities of dry matter and nitrogen were not affected.

In response to concerns that consumption of the milk containing increased concentrations of volatile fatty acids might affect human health adversely, Papas and Sniffen (97) reported no increased concentration of volatile fatty acids in milk. For these reasons, ammonium or calcium salts of volatile fatty acids have been fed in the commercial farms in recent years.

EFFECTS OF DIETARY FATS ON RUMEN FERMENTATION

Mono and digalactoglycerides, triglycerides, and phospholipids in feed are hydrolyzed into galactose, glycerol, and fatty acids by ruminal bacteria in the rumen (9). Several rumen bacteria and protozoa (9) rapidly hydrogenate unsatu-

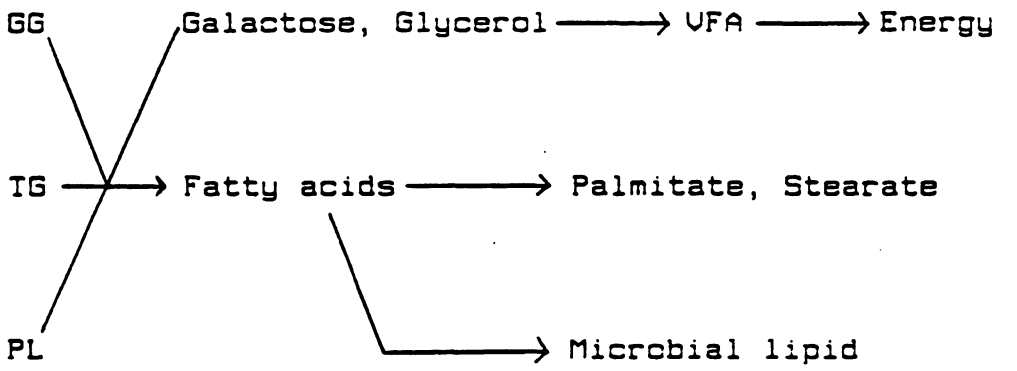

Figure 2. Lipid fermentation by microbes in the rumen.
GG = Galactoglycerides, TG = Triglycerides
PL = Phospholipid, VFA = Volatile fatty acid
Adapted from (76).

rated fatty acids into saturated fatty acids (9, 76, 91), to balance excessive reducing equivalents produced by ruminal bacteria (76). According to Palmquist (91), the biohydrogenating process appears to play an important role in protecting ruminal bacteria from toxic effects of polyunsaturated long-chain fatty acids. Unsaturated fatty acids are more toxic to ruminal bacteria than saturated fatty acids, due to their high solubility and reactivity in the rumen (40).

Also, volatile fatty acids are produced by the microbial activity, transported through the blood stream, and utilized for energy (60). The acidity of fluids in the duodenum and upper jejunum is higher in ruminants than in nonruminants (76, 95). This higher acidity enables saturated fatty acids to be further digested (95) and enables protected lipids (i.e., formaldehyde-treated lipid-casein complexes or calcium soaps) to be easily dissociated in the lower digestive tract of cows (10).

The metabolism of unsaturated fatty acids seems to be important because they are major components of dietary fatty acids, and are further saturated in the rumen (106). To avoid ruminal hydrolysis and biohydrogenation of fat, protected lipid feeding has been recommended with allowing the production of more unsaturated milk and meat. However, the effects of dietary fats on rumen fermentation remain

still unclear (91).

Palmquist et al. (91, 95) has reported that dietary fats did not influence rumen fermentation. However, in an in vitro fermentation study (23) of long-chain fatty acids, the acetate to propoinate ratio was reduced when 15 and 20% substrates consisted of long-chain fatty acids. This change in components of volatile fatty acids has been observed to be a result of antibacterial activities of both unsaturated and saturated long-chain fatty acids (40, 91, 95). According to an in vitro study of Galbraith et al. (40), long-chain fatty acids inhibit gram positive bacteria, and not gram negative bacteria. Antibacterial activities were highest in the order of unsaturated fatty acids (18:3>18:2>18:1>18:0), lauric, myristic, palmitic, and stearic acids. Calcium ions alleviated these toxic effects. Negative effects of long-chain fatty acids have been frequently observed in unprotected fat diets (8, 34, 100, 106, 122).

In two studies (9, 76), the acetate to propionate ratio was positively correlated to the production of milk and milk fat. Fat synthesis was highly dependent on acetate and butyrate production in the rumen (76). According to an in vivo study (60), acetic acids infused into the rumen increased yields of milk and milk fat, but propionic acids decreased them while increasing milk protein content.

In conclusion, the depressed milk fat caused by feeding unprotected fats may result possibly from the negative effects of long-chain fatty acids on rumen fermentation.

EFFECTS OF DIETARY FATS ON DIGESTIBILITIES

Increased digestibilities are accompanied by increased dry matter and energy intake, and increased animal performance (26). The effects of dietary fats on digestibilities of ruminants are not clearly established. Growth of ruminal cellulolytic bacteria was highly inhibited by long-chain fatty acids, primarily oleic acid in high fat diets (91). For this reason, unprotected fat feeding tended to decrease fiber digestibility (34). When fiber digestion is depressed, less acetate is produced and consequently, production of milk and milk fat is depressed (60, 76).

Palmquist (91) has suggested that calcium addition to fat diets may reverse the depressed fiber digestion because calcium ions combined with fatty acids in the rumen form insoluble soaps which would escape rumen fermentation. This worker has further recommended to feed higher amounts of fiber to offset the depressed fiber digestion (91). Likewise, it has been known that protein-coated fat or whole cottonseed as protected lipids can avoid rumen fermentation

and allow digestibilities to be normally maintained.

BLOOD METABOLITE RESPONSE TO DIETARY FAT

Milk synthesis depends highly upon the rate of blood flow because the blood circulation delivers nutrients from tissues to mammary gland (27). In early lactation, blood non-esterified fatty acid concentration rises due to fat mobilization (123), and glucose is less used than lipids as energy by body tissues (26).

Palmquist (90) correlated dietary fat intake with high concentration of plasma total lipid. Similarly, Mata-Hernandez et al. (74) has reported that dietary protected fat increased the levels of plasma lipid.

Hove (55) has suggested that plasma ketones showed a large variation in healthy cows, and that lower plasma glucose in early lactation is probably due to its high utilization for milk synthesis. McAdam et al. (77) have shown large fluctuations of concentrations of plasma calcium and magnesium as lactation proceeded.

All the nutrient partitioning and energy metabolism occur under the homeostatic and homeorhetic control of hormones (15). Growth hormone stimulates lipolysis, and insulin stimulates lipogenesis in the adipose tissues (26, 78, 91).

After a meal, ratio of insulin to glucagon is increased (15). The interactions between growth hormone, insulin, and glucagon must be associated with nutrient partition and maximal milk production (26). By data of Hart et al. (47), blood growth hormone concentration and growth hormone to insulin ratio were positively associated with milk yield. Growth hormone was negatively correlated with body weight gain and positively with plasma non-esterified fatty acids. In early lactation, concentration of plasma growth hormone increased, that of plasma insulin decreased, (52, 123) and growth hormone to insulin ratio increased (52, 91).

Epinephrine also has been known to stimulate lipolysis and increase plasma non-esterified fatty acids (15), especially in early lactation (17). It is more active for humans, rats, and dogs than for cows (76). Jaster et al. (56) found that lipolysis by epinephrine may be highly associated with cyclic AMP concentration and the number of beta-adrenergic receptors in adipose tissues.

In addition, It has been reported that intravenously administered short-chain fatty acids and amino acids increased lipogenesis and gluconeogenesis, respectively, resulting from the stimulation of insulin (78).

Ketogenesis is highly associated with hypoglycemia. Intravenously injected glucose induced higher insulin

activity and greatly decreased concentrations of plasma ketones (46, 114). Metabolism of glucose may be altered by either high-grain intake (36) or dietary fat supplement (31). Glucose pool size was highly dependent on concentrations of plasma glucose (36), and feed intake (22). Bartley et al. (13) has suggested that as volatile fatty acid concentrations in the rumen increased, plasma glucose concentration decreased, resulting in insufficient supply of glucose to the mammary gland for milk production. He has further reported that endogenous glucose metabolism was inhibited by exogenously injected glucose (13).

LACTATIONAL EFFECT OF DIETARY FAT

The daily production of milk fatty acids is greater than intake of fatty acid; so it is important to understand the metabolism of milk fat synthesis in the mammary gland (95). Over 95% of milk lipid is composed of triglycerides, and the lipid content has shown the most variation among milk components (82). Unprotected fat reduces fiber digestion in the rumen (91). Consequently, ratio of acetate to propionate was reduced, and milk fat was depressed (50, 58, 75, 95). It has been suggested that as greater amounts of

dietary long-chain fatty acids were transfered to milk fat, percentages of long-chain fatty acids in the milk increased and those of short-chain fatty acids decreased (44, 75, 91, 95, 102). Mattos et al. (75) reported that the primary reason for milk fat depression was the inhibited de novo fatty acid synthesis in the mammary gland. Plowman et al. (99) postulated that feeding unprotected safflower oil-casein increased the hydrogenation of linoleic acids to oleic acids, resulting in high content of oleic acids in the milk. Palmquist et al. (91) confirmed that the increased oleic acids in the milk were attributed to desaturation of stearic acids in the mammary gland.

Gerson et al. (42) suggested that oleic acids in the milk may be derived from caprylic (8:0), capric (10:0), and lauric (12:0) acids in the mammary gland. However, in protected oil, the uptake of 18-carbon fatty acids may be inhibited by dietary polyunsaturated fatty acids (20:5 to 22:6) (90).

The source of long-chain fatty acids in the milk is blood glyceride (42) absorbed from dietary fat (82), and that of short-chain fatty acids is blood acetate (42) serving as precursors for de novo synthesis of short-chain fatty acids (82). The produced fatty acids are further utilized for milk fat synthesis (82, 112). Also, chylomicron cholesterol may be absorbed by the mammary gland through the hydrolytic

activity of lipoprotein lipase (82).

By feeding fat, milk butyric acid content increased (24, 75, 102, 112). Chandan et al. (24) suggested that the increased butyric acid may be attributable to its selective release by pancreatic lipase and milk lipase. Storry et al. (112) has proposed that there may be a non-malonyl pathway for butyrate synthesis.

In general, the milk fat depression observed when feeding unprotected lipids can be restored by either substituting protected lipids (8, 44, 75, 106, 113, 126) or by adding more fiber (50) or calcium (51, 57, 58, 64, 96). For protected lipids, percent milk fat was increased because greater contents of long-chain fatty acids were synthesized than the reduced amount of short-chain fatty acids (75). However, milk protein content was depressed probably due to decreased casein nitrogen (33, 42).

Smith et al. (110) has suggested that depressed milk lactose, and solids-not-fat may be caused by the restricted supply of glucose to the mammary gland.

LIPOLYSIS AND LIPOGENESIS IN ADIPOCYTES

The physiological adaptation of lipolysis and lipogenesis occurs under endocrine regulation (27). During early

lactation, the rate of lipolysis in adipose is high and that of lipogenesis is relatively low, due to extremely high demand of energy for milk synthesis (15, 26, 27, 83). The key tissues related to lipid metabolism are mammary and adipose tissues. The metabolic adaptation related to lipogenesis (predominently in late lactation) and lipolysis (in early lactation) are indicated in Figure 3.

Lactogenesis increases the rate of lipolytic activities (Figure 3. B) in adipose cells (15, 26, 27, 83). The released glycerols and free fatty acids combined with blood albumin are transported to the mammary gland (15, 27, 83) with increased blood flow rate (27). The glycerol release into the blood is an irreversible reaction because cows do not have glycerol kinase in adipose tissue (83). The released nutrients are delivered to the mammary gland directly or indirectly via liver (27). Lipolysis in adipocytes is stimulated by reduced activities of acetyl CoA carboxylase (15) and lipoprotein lipase (27), reduced insulin receptors, increased albumin concentrations in blood (16), and increased beta-adrenergic receptors (27, 56).

Metz et al. (83) has observed that as fat mobilization increased, in vitro re-esterification of fatty acids almost stopped. In an in vitro study by Yang et al. (129), fat mobilization was stimulated by infusion of 3-hydroxybutyrate

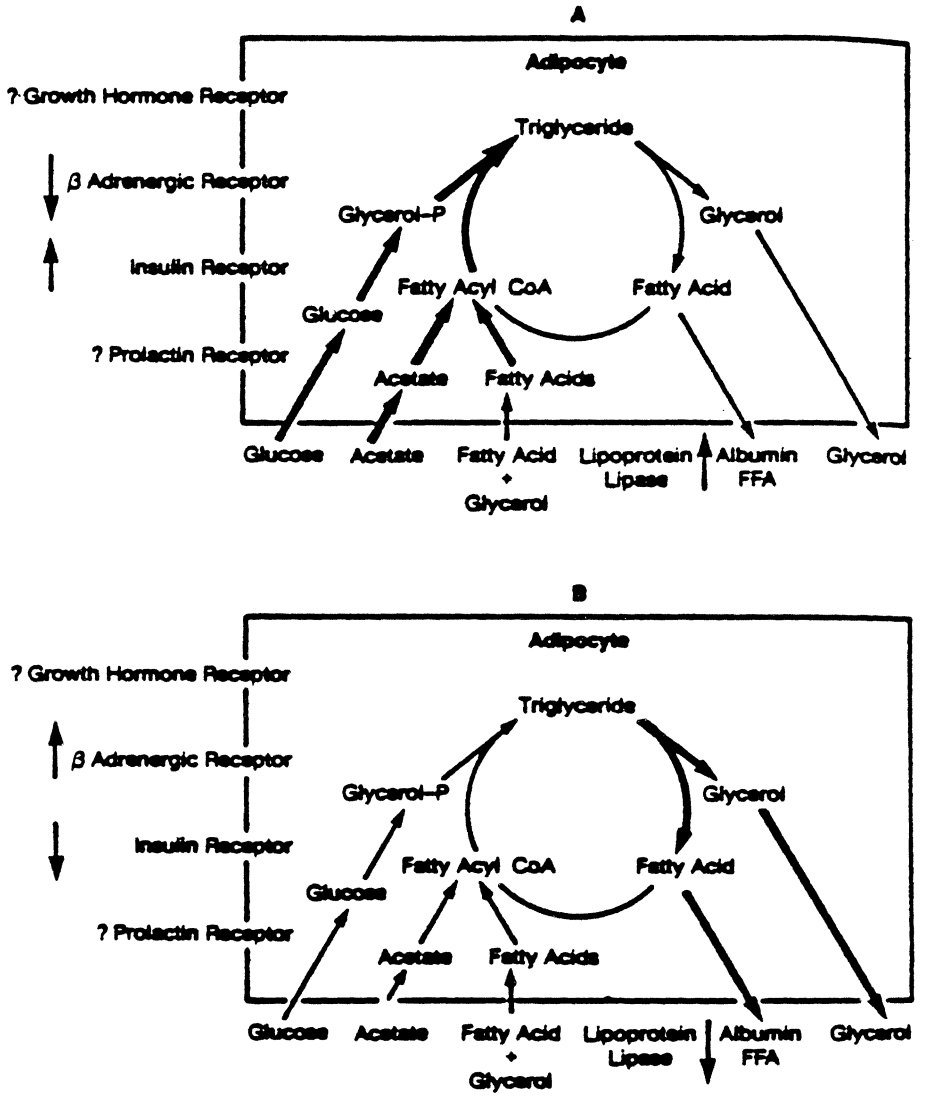

Figure 3. Lipogenesis (A) and lipolysis (B) in adipose. Taken from (27).

and depressed by glucose, insulin, and nicotinic acid. Metz et al. (83) found high sensitivity of lipolysis to noradrenalin. Conversely, lipogenesis remains high during late lactation when energy balance is positive (15, 26, 27). In conclusion, to understand the interactions between fat, fiber, metal cations, and microflora in the rumen (95), more efforts should be made.

MATERIALS AND METHODS

SELECTION AND FEEDING REGIME OF ANIMALS

Forty four first lactation Holstein cows were randomly housed in tie stalls within 3 days after calving, fed control diet (Table 1), for 14 days for adjustment and standardization. Cows were ranked by milk production during the second week of lactation and assigned to 8 dietary groups, such that average milk production of groups was similar. Twenty four cows were assigned to 4 dietary groups: 1) control diet (C) with 35.2% corn silage, 14.4% alfalfa haylage and 50.4% concentrate (dry matter basis); 2) C + 2% calcium stearate (S); 3) C + 2% tallow (T); and 4) C + 10% whole cottonseed (W). Fat supplements were substituted for corn grain, and whole cottonseed for corn grain, cottonseed meal and cottonseed hulls. The remaining 20 cows were assigned to 4 additional dietary groups: C + isoacid (CI), S + isoacid (SI), T + isoacid (TI) and W + isoacid (WI). Four g isoacid[1] as fed basis per kg dry matter was added. All the experimental diets were fed for 4 weeks. The ingredient and

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[1]IsoPlus, Eastman.

chemical composition of complete mixed diets are presented in Table 1 and Table 2, respectively.

COLLECTION OF SAMPLES

Cows were fed at 0600 and 1400 h and milked at 0030 and 1230 h. From week 2 to week 6, feed refusals were determined on four consecutive days weekly. Body weights and milk weights were recorded twice weekly. Milk samples were collected in plastic bags containing 2 potassium dichromate tablets (NASCO), stored at room temperature, and analyzed for percentages of milk fat, protein, lactose, and solids-not-fat (SNF) within 48 hours. Somatic cell count (SCC) also was analyzed. Samples of forage and concentrates were collected every other week throughout the experiment, and divided into four portions. One portion was sent to the Virginia Tech Forage Testing Laboratory for analysis. A portion was dried at 100°C for 24 hours. The other two portions were dried at 60°C for 48 hours and ground through a 1 mm Wiley Mill screen for analysis. One portion was analyzed in the dairy nutrition laboratory and the other sent to New York Forage Testing Laboratory for analyses of crude protein (CP), acid detergent fiber (ADF), calcium, phosphorus, and magnesium.

Fecal grab samples were taken at the end of weeks

Table 1. Composition of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

Ingredient[a,b]	C	CI	S	SI	T	TI	W	WI
Corn silage	35.2	35.2	35.1	35.1	35.0	35.0	35.2	35.2
Alfalfa haylage	14.4	14.4	14.3	14.3	14.3	14.3	14.4	14.4
Cottonseed meal	5.3	5.3	5.3	5.3	5.2	5.2	---	---
Cottonseed hulls	4.1	4.1	4.1	4.1	4.1	4.1	---	---
Corn grain	27.0	27.0	25.1	25.1	25.1	25.1	26.4	26.4
Soybean meal (44%)	13.2	13.2	13.5	13.5	13.4	13.4	13.2	13.2
Dicalcium Phosphate	.11	.11	.12	.12	.11	.11	.11	.11
Limestone	.59	.59	.20	.20	.58	.58	.59	.59
Trace mineral salt	.26	.26	.26	.26	.26	.26	.17	.17
Calcium stearate[c]	---	---	2.0	2.0	---	---	---	---
Tallow[d]	---	---	---	---	2.0	2.0	---	---
Whole cottonseed	---	---	---	---	---	---	10.0	10.0
IsoPlus[e]	---	.32	---	.32	---	.32	---	.32

[a] % of dry matter.

[b] Vitamin A, 6000 IU/kg; Vitamin D, 600 IU/kg; Vitamin E, 25mg/kg.

[c] SYNPRO 24-46, Synthetic Products Company, Cleveland, OH. Composition: 65% calcium stearate, 30-35% calcium palmitate, 0-5% calcium myristate, 6.5% calcium.

[d] Stabilized yellow grease, Carolina By-product Company, Inc, Greensboro, NC.

[e] Eastman Chemical Products, Inc. Kingsport. Tennessee 37662. Composition: 80% (min.) calcium salts of isobutyric and mixed 5-carbon volatile fatty acids, 3% (max.) calcium hydroxide, 13.8% (min.) calcium, and 17% (max.) water.

Table 2. Nutrient composition of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

Nutrient[a]	C	CI	S	SI	T	TI	W	WI
Crude protein[b]	17.3	17.4	17.3	17.3	17.2	17.2	17.2	17.3
Acid detergent fiber[b]	20.4	20.6	20.3	20.9	20.4	20.7	20.9	20.8
Calcium[c]	.60	.62	.64	.64	.60	.65	.64	.64
Phosphorus[c]	.41	.42	.43	.42	.41	.41	.41	.43
Magnesium[c]	.25	.25	.26	.25	.24	.25	.25	.26
NE , Mcal/kg[d]	1.64	1.64	1.71	1.71	1.71	1.71	1.73	1.73

[a]% of dry matter.

[b]Analyses by N.Y. and U.T. forage testing laboratories.

[c]Analysis by N.Y. forage testing laboratory.

[d]Net energy of lactation was calculated using (9).

2 and 6, dried at 60°C for 48 hours, ground through a 1 mm Wiley Mill screen, and stored for analyses of CP, ADF, and acid detergent fiber insoluble ash (ADFIA). Rumen fluid samples obtained at the end of weeks 2 and 6, were frozen at -20°C and stored for future analysis of volatile fatty acids (VFA).

Jugular blood samples were taken into 30 ml syringes. Two 10 ml aliquots were placed into centrifuge tubes containing 200 IU heparin in 100 ul saline. Two 5 ml aliquots were placed in tubes containing 5 ml 10% perchloric acid for deproteinization. Immediatedly after collection, samples were placed in ice, transported to the laboratory, and centrifuged at 3000 x g for 20 min. Plasma was decanted and stored at -20°C until analysis for non-esterified fatty acids (NEFA), glucose, growth hormone, insulin, calcium, and magnesium. Deproteinized whole blood also was decanted and stored at -20°C until analyzed for acetoacetate and D-3-hydroxybutyrate.

EPINEPHRINE AND GLUCOSE LOADING REGIME

Cows fed C, S, T, and W were used to evaluate responses to epinephrine and glucose injections during the last week of the feeding trial. A catheter was placed in the right or

left jugular vein 24 hours before sample collection. Cows were placed in a stanchion and given an intravenous injection of 1 to 1.25 ml of Rompun (10 mg/ml). After jugular blood vessel penetration with a 13 gauge needle, a polyethylene catheter (CAT. No. 602-285, SILASTIC, DOW CORNING) was installed. A stub adapter (No. 7561, 16 gauge, CLAY ADAMS) and an injection cap (No. 6974, BECTON- DICKINSON) were attached to seal the end of the catheter. Heparinized-saline (340 IU/ml) was injected to prevent blood clotting, and the free end of the catheter attached to the neck with tape. Injections of epinephrine and glucose were given on consecutive days and separated by 24 hours between injections. Samples were collected at -15, -10, -5, 0, 3, 6, 9, 12, 15, 20, 25, 30, and 40 minutes relative to injection of 0.7 ug epinephrine or 0.1 g glucose per kg body weight. Samples were placed in crushed ice, and centrifuged at 3000 x g for 20 minutes. Duplicate aliquots of plasma were stored at -20°C for analyses of NEFA and glucose, respectively.

MILK AND PLASMA ANALYSES

Percent milk fat, protein, lactose, and SNF were determined by Infrared Milk Analyzer, and somatic cell counts

per ml measured by Milko-Tester[2] in the DHIA laboratory.

Plama NEFA concentration was determined in triplicate by the acyl CoA synthetase-oxidase method as indicated in the WAKO manual (1), using a split-beam spectrophotometer.[3] Concentrations of the standard solutions were 0, 125, 250, 500, and 1000 uEq/l. To avoid variation due to low concentrations of NEFA, 500 uEq/l of standard solution was added to each sample. Absorbance was recorded at 550 nm and converted to concentration by the linear regression of the standard curve. NEFA concentration of samples was calculated by subtracting 500 uEq/l.

Glucose concentration in plasma was determined in triplicate and analyzed according to Sigma Technical Bulletin, No. 510. Absorbance was determined at 450 nm using the same spectrophotometer noted above.

Calcium and magnesium concentrations in plasma were analyzed in duplicate with an atomic absorption spectrophotometer[4] according to the procedure in (3).

Concentrations of growth hormone and insulin in plasma were quantified in triplicate by a double antibody radioimmunoassay (7).

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[2]FOSSOMATIC, A/S N. FOSS ELECTRIC, DENMARK.

[3]Bausch & Lomb Spectronic 1001.

[4]Parkins-Elmer Model 370.

Deproteinized blood samples were neutralized with 20% potassium hydroxide for the determination of blood acetoacetate and D-3-hydroxybutyrate. Reduced nicotinamide-adenine dinucleotide (NADH) concentration was determined at 365 nm on a Beckman spectrophotometer (Model 35) using Mellanyby and Williamson's enzymatic analysis methods (81), and multiplied by specific dilution factor and extinction coefficient to calculate each ketone concentration, uM. The total concentration of blood ketone was obtained by adding blood acetoacetate to D-3-hydroxybutyrate concentration.

RUMEN VOLATILE FATTY ACID ANALYSIS

The thawed 5 ml of rumen fluid was mixed with 1 ml of 25% metaphosphoric acid and 5 ml of isocaproic acid (internal standard) and centrifuged at 3000 x g for 10 minutes to remove particulates. Samples were analyzed in duplicate. The standard solution was prepared in the same manner. Concentrations of acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids were quantified using gas chromatography[5] with built-in integrator. Units were

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[5]Vista Series, Varian.

uMoles/ml.

DIGESTIBILITY COEFFICIENT

Composition of diets was obtained by averaging our data, data from VT Forage Testing Laboratory, and New York Forage Testing Laboratory. Fecal composition was determined in the dairy nutrition laboratory. Apparent digestibility coefficients for dry matter, crude protein, and acid detergent fiber were determined using acid detergent fiber insoluble ash as an indicator.

Feed and fecal samples were analyzed in duplicate. Percent crude protein was determined using Kjeldahl[6] analysis. Percent acid detergent fiber was quantified according to VanSoest's method (43, 121). Acid detergent fiber insoluble ash was determined using the residue of the acid detergent fiber assay. Samples were burned at 600°C for 3 hours and then at 250°C for 30 minutes. Digestibility coefficient of dry matter was calculated using Equation 1. Digestibility coefficients of crude protein and acid

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[6]KJELTEC SYSTEM, 1002 DISTILLING UNIT, and DIGESTION SYSTEM 20, TECATOR, SWEDEN.

detergent fiber were obtained by Equation 2.

Equation 1.

$$DC = 100\% \frac{(FeI)(FcN)}{(FcI)(FeN)}$$

Equation 2.

$$DC = 100\% - 100\% \frac{(FeI)(FcN)}{(FcI)(FeN)}$$

where DC = Digestibility coefficient

FeI = Feed indicator

FcI = Feces indicator

FcN = Feces nutrient

FeN = Feed nutrient

DETERMINATION OF GLUCOSE PARAMETERS AND PLASMA NEFA RESPONSE TO EPINEPHRINE

Samples from glucose and epinephrine challenges were analyzed in the same manner noted for plasma glucose and NEFA, respectively. Post-injection concentrations of glucose(mg/dl) and NEFA(uEq/l) in plasma were recorded as total minus basal concentration, estimated by averaging -15, -10, -5, and 0 min concentrations.

The 40 min glucose sample was omitted from calculations, because concentration had reached basal level. Concentrations (y) transformed into natural logs fit a linear curve plotted against time (t). Slope and intercept of each curve were estimated by regression analysis. Glucose clearance rate was calculated by multiplying the negative slope by 100 and recorded as %/min. Pool space was estimated by dividing the injected dose (g) by the antilog of the intercept and expressed as liters. Pool size (g) was calculated by multiplying the basal concentration (g/l) by pool space (liter).

The peak time of plasma NEFA response to epinephrine loading was estimated from a fitted polynomial regression. The increased plasma NEFA concentration (area) was calculated

by integration.

STATISTICAL ANALYSES

All blood, milk, feed intake, and weight gain data were subjected to two way analysis of covariance using the general linear model (GLM) of SAS (6) shown in Model 1.

Model 1. Two way analysis of covariance

$$Y_{ijk} = u + d_i + w_j + d{*}w_{ij} + b(X_{ilk} - \bar{X}_{.1.}) + e_{ijk}$$

where Y_{ijk} = observation of kth cow fed ith diet in jth week (j=2, 3, 4, and 5).

u = grand mean of population

d_i = diet effect for group i

w_j = jth week effect

$d{*}w_{ij}$ = interaction effect of ith diet by jth week

$b(X_{ilk} - \bar{X}_{.1.})$ = effect explained by the difference of X_{ilk} from $\bar{X}_{.1.}$ (covariant)

e_{ijk} = error term

All digestibility and volatile fatty acid data were analyzed by one way analysis of covariance (Model 2) due to absence of a week effect. Data from 14 day standardization period were used as the covariate. Orthogonal contrasts among diets were tested (ie. control diet versus fat supplemented diets etc).

Model 2. One way covariance of analysis

$$Y_{ij} = u + d_i + b(X_{i1} - \bar{X}_{.1}) + e_{ij}$$

Plasma NEFA response to epinephrine infusion was analyzed with a split plot design (Model 3). The fitted curve of plasma NEFA response to epinephrine infusion was estimated using polynomial regression indicated in Model 4. Glucose clearance rate, pool space, pool size, and acetate to propionate ratio were subjected to one way analysis of variance. All data were tested at the .05 level of significance.

Model 3. Split plot design

$$Y_{ijk} = u + d_i + c_{j(i)} + t_k + d*t_{ik} + e_{ijk}$$

where Y_{ijk} = observation of kth time of jth cow fed ith diet

d_i = ith diet effect

$c_{j(i)}$ = effect for jth cow fed ith diet

t_k = time (min.) effect

$d*t_{ik}$ = interaction effect of ith diet by kth time (min.)

e_{ijk} = error term

Model 4. Polynomial regression

$$Y = a + b_1 X + b_2 X^2 + b_3 X^3 + b_4 X^4 + E$$

where Y = plasma NEFA concentration at X minute

a = intercept

b_i = regression coefficient

X = minute

E = error term

RESULTS

DRY MATTER INTAKE, MILK YIELD, WEIGHT GAIN, AND EFFICIENCY OF UTILIZATION OF ENERGY

Fat supplementation did not significantly affect dry matter intake (Table 3); however, isoacid addition significantly increased dry matter intake of all diets, especially W. Dry matter intake (Figure 4) increased over the experimental period; however, W, WI, T, and TI-fed cows tended to decrease intake near the end of the experimental period. Milk yield (Table 3) was not significantly affected by dietary treatments; however, average yield was highest for CI- and S-fed cows and lowest for W. Milk yields of CI, S, and WI groups (Figure 5) were consistently higher than that of group C.

Weight gain (Table 3) was affected by dietary treatments. Cows fed C and WI diets gained weight over the experiment. Isoacid addition to C decreased weight gain and isoacid addition to W increased gain. Fat supplemented groups (S, T, and W) had significant net weight losses compared to net weight gain for group C; however, they started to gain weight gradually from the third week to the end of experimental

Table 3. Dry matter intake, milk yield, weight gain, and efficiency of energy utilization associated with the feeding of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

	C	CI	S	SI	T	TI	W	WI	SE
Dry matter intake, kg/day	14.9	15.5	15.2	15.6	14.9	15.6	14.9	16.5[f]	.4[g]
Milk yield, kg/day	26.6	27.7	27.7	26.3	27.0	26.4	26.3	27.1	.6
Weight gain, kg	7.3[a]	-8.7[c]	-2.8	-7.8	-5.5	-8.9	-8.5	3.9[f]	3.7
Efficiency of energy[h] utilization, %	71.4[a]	68.9	70.0	67.8	69.1	67.0	61.9	66.4	2.3

[a]contrast C vs. S, T, W; p<.05.

[c]contrast C vs. CI; p<.05.

[f]contrast W vs. WI; p<.05.

[g]contrast C, S, T, W vs. CI, SI, TI, WI; p<.05.

[h]calculated using (119).

period (Figure 6). Net weight gain for TI was greatly reduced during the last two weeks of experiment. Efficiency of utilization of dietary energy for milk production (Table 3) was highest for C and lowest for W. Isoacid addition had no significant effect on efficiency.

MILK COMPONENTS

Milk constituents are listed in Table 4. Percent milk fat was highest for SI and lowest for W. Dietary fat supplementation significantly depressed milk fat percent primarily due to the large depression in the W-fed group. Isoacid addition significantly enhanced milk fat percent of groups fed dietary fat, but not C-fed cows. SI and WI had significantly higher milk fat percent than S and W, respectively, due to the negative effect of fat supplementation and the positive effect of isoacid addition. Percent milk fat (See Appendix, Figure 8) of almost all groups except C tended to decrease during the last week of experiment. This decrease in milk fat percent occurred when body weight began to increase.

Milk protein response was not significant for any dietary treatment except isoacid addition to the control diet. CI was significantly lower than C. It has been suggested that

Table 4. Milk constituents associated with the feeding of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

	C	CI	S	SI	T	TI	W	WI	SE
Fat %	3.40[a]	3.35	3.18	3.62[d]	3.21	3.47	3.02	3.41[f]	.13[g]
Protein %	2.99	2.88[c]	2.91	2.95	2.99	3.01	2.97	3.00	.04
Lactose %	5.26[a]	5.12[c]	5.09	5.00	5.06	5.29[e]	4.83	5.05[f]	.06
Solids-not-fat %	9.30[a]	9.03[c]	9.04	9.04	9.06	9.29[e]	9.00	9.10	.03
Somatic cell counts[h]	1.32	1.17	2.71	.70	2.66	1.48	1.50	2.03	.95

[a]contrast C vs. S, T, W; $p<.05$.

[c]contrast C vs. CI; $p<.05$.

[d]contrast S vs. SI; $p<.05$.

[e]contrast T vs. TI; $p<.05$.

[f]contrast W vs. WI; $p<.05$.

[g]contrast C, S, T, W vs. CI, SI, TI, WI; $p<.05$.

[h]in cells $\times 10^3$/ml.

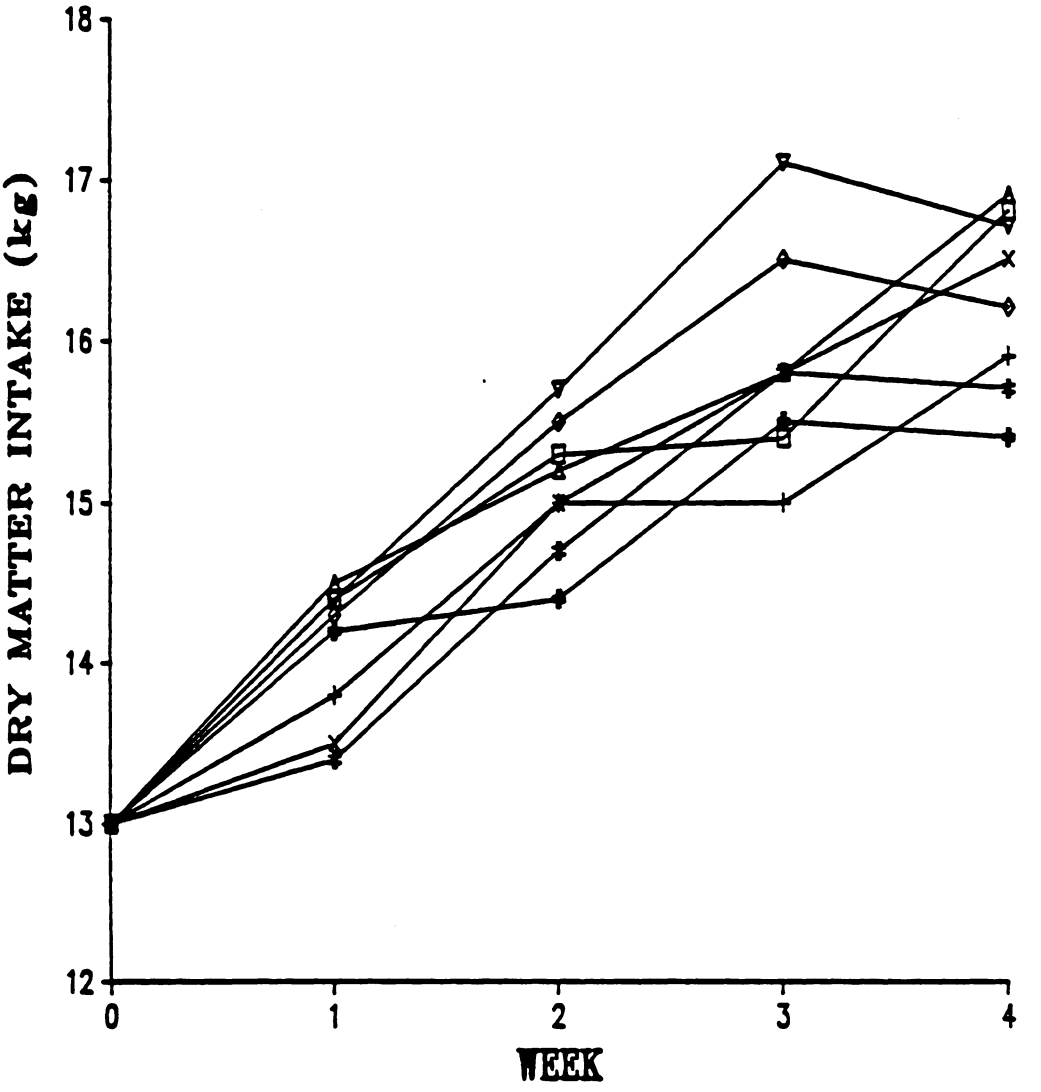

Figure 4. Dry matter intake of cows fed control (+), calcium stearate (×), tallow (✱), and whole cottonseed (✱) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

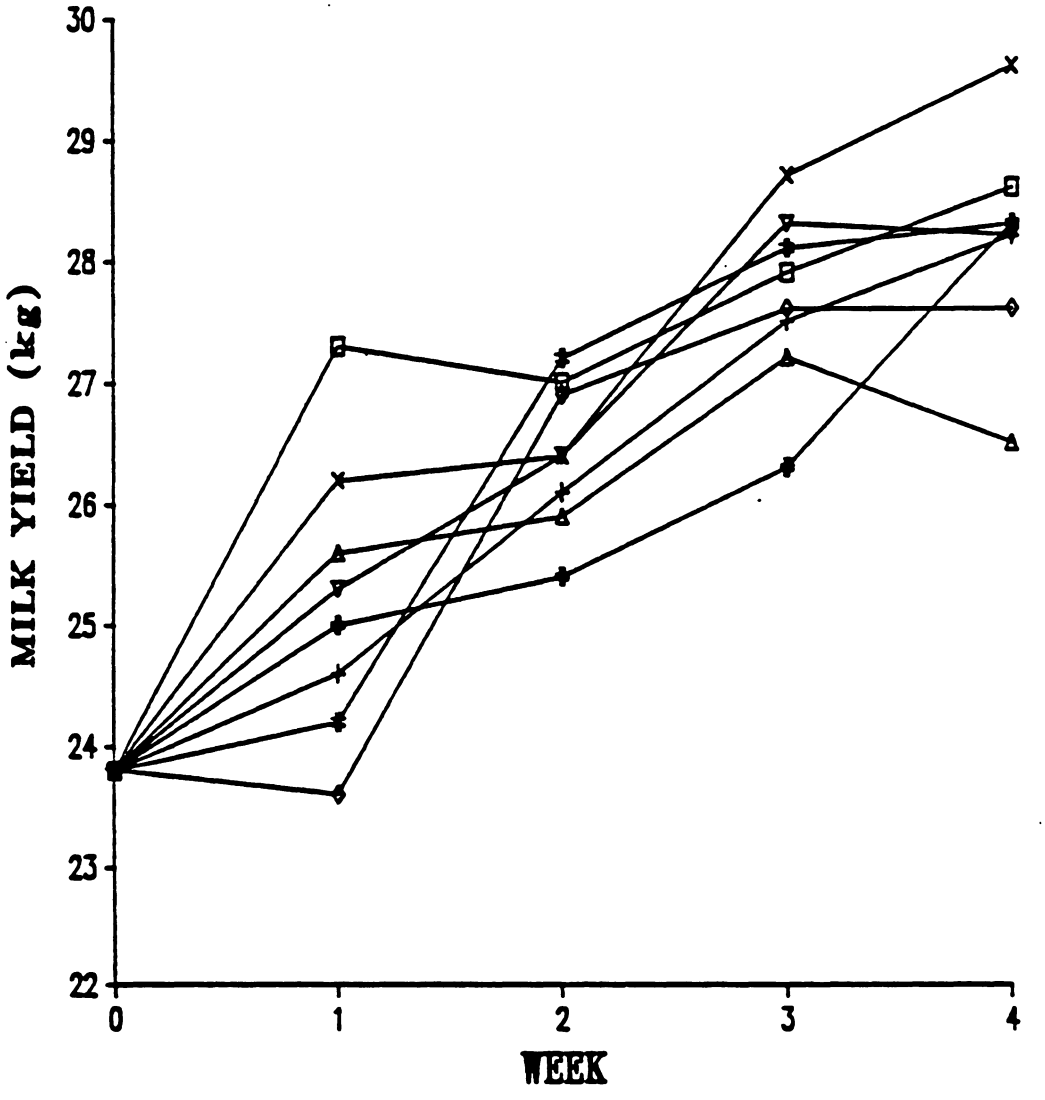

Figure 5. Milk yield of cows fed control (+), calcium stearate (×), tallow ($), and whole cottonseed (ϕ) diets, and control (□), calcium stearate (Δ), tallow (◊), and whole cottonseed (▽) diets with added isoacids.

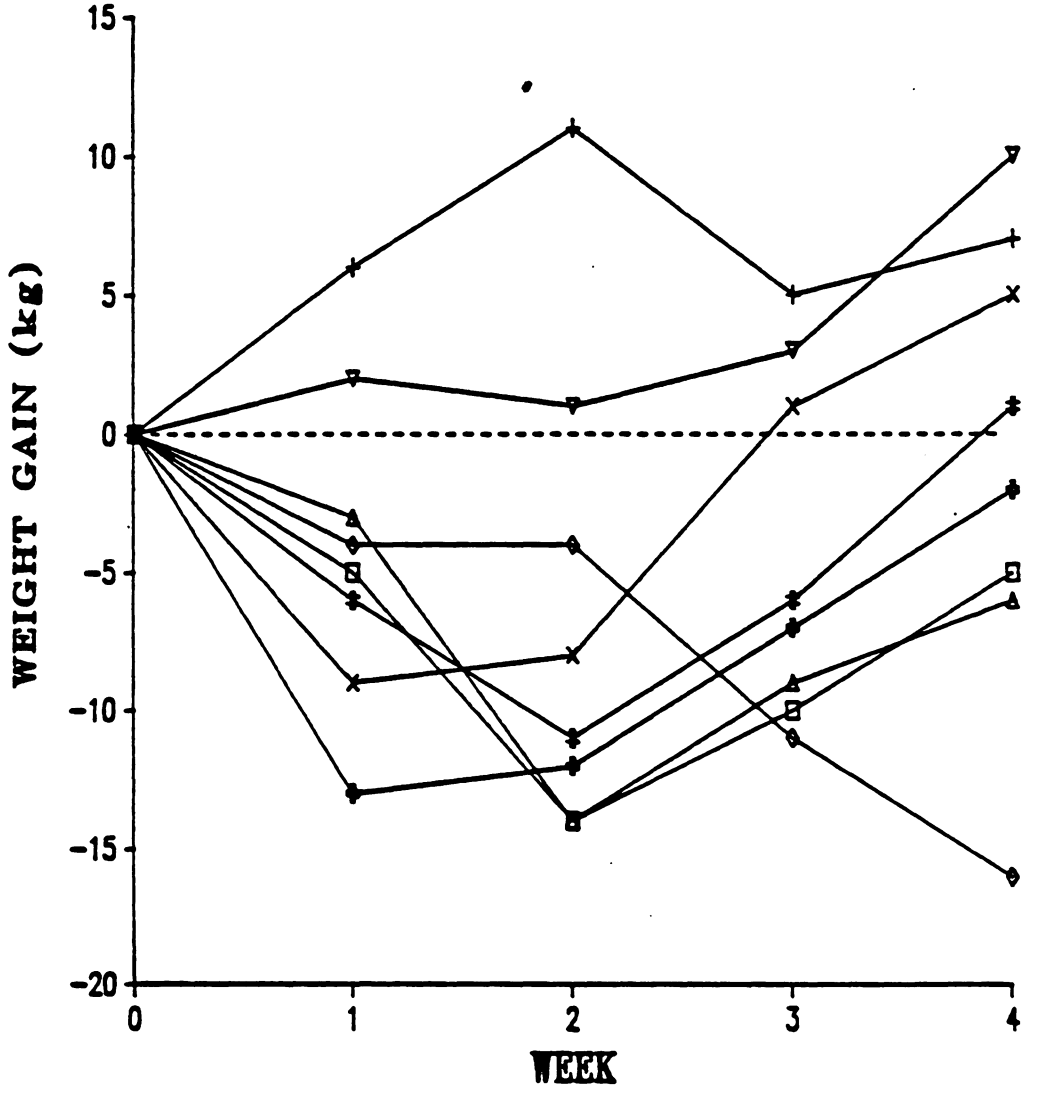

Figure 6. Weight gain of cows fed control (+), calcium stearate (X), tallow (I), and whole cottonseed (Φ) diets, and control (□), calcium stearate (Δ), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

protected fat supplements may depress milk protein (33, 91), but such a response was not noted in this study. Lactose synthesis in the mammary gland depends on the availability of glucose. Fat supplements significantly reduced milk lactose percent. All groups except TI had low lactose in milk compared to C over the experimental period (See Appendix, Figure 10). Isoacid in CI reduced lactose; whereas, isoacid in TI and WI elevated lactose.

Milk solids-not-fat percent is highly dependent upon protein and lactose concentrations in milk. The pattern of solids-not-fat response was similar to that of lactose response, except that the increase for WI was not significant.

There were no significant differences of somatic cell count due to diets in this study.

Yields of major milk constituents and 4% fat-corrected milk are in Table 5. Milk fat yield varied primarily with percent milk fat. Isoacid additions significantly increased milk fat yield primarily due to increased milk fat percentage. Milk fat yield for W decreased markedly over the experimental period. During the fourth week, milk fat yields of all isoacid-added groups decreased; whereas, those of all groups without added isoacids increased (see Appendix, Figure 12).

Table 5. Yields (kg/d) of milk constituents and fat-corrected milk associated with the feeding of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

	C	CI	S	SI	T	TI	W	WI	SE
Fat	.89	.90	.87	.95	.84	.90	.79	.92[f]	.04[g]
Protein	.79	.76	.79	.77	.79	.78	.78	.81	.02
Lactose	1.39	1.37	1.41	1.34	1.41	1.37	1.19	1.39[f]	.04
4% fat-corrected milk	23.5	24.2	24.0	24.8	23.2	24.3	22.3	24.5[f]	.5[g]

[f]contrast W vs. WI; $p<.05$.

[g]contrast C, S, T, W vs. CI, SI, TI, WI; $p<.05$.

Milk protein yields were similar for all diets and increased gradually over time (see Appendix, Figure 14). Milk lactose yield was affected only by isoacid addition to W, due to the low yield by cows fed W (see Appendix, Figure 15). Despite reduced percent milk protein and lactose over time, yields of milk protein and lactose increased with time primarily due to the elevated milk production.

Fat-corrected (4%) milk is highly dependent upon change in milk fat percentage. It increased over time (see Appendix, Figure 13). Overall, isoacid addition resulted in an average of 1.1 kg/day more 4% fat-corrected milk; however, the difference between W and WI was the only significant individual response. Cows fed SI responded positively and steeply from the first week and cows fed C yielded the highest fat-corrected milk at the end of the experimented period. The group fed W had the lowest yield of fat-corrected milk.

BLOOD METABOLITES AND HORMONES

Selected blood metabolites and hormones are presented in Table 6. Concentrations of non-esterified fatty acids (NEFA) in plasma reflect rate of fatty acid mobilization from adipose tissue, rate of fatty acid utilization, and possibly

Table 6. Jugular whole blood and plasma metabolites associated with the feeding of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added iscacids.

	C	CI	S	SI	T	TI	W	WI	SE
Non-esterified fatty acids, uEq/l	138[a]	122	144	95[d]	99	128	87	114[f]	12
Glucose, mg/dl	62.4	60.4	60.3	62.5	62.2	59.6	61.2	65.1	1.7
Calcium, mg/dl	9.32	9.34	9.38	9.69	9.29	8.92	9.14	9.38	.21
Magnesium, mg/dl	2.04	1.87[b]	2.17	2.21	2.04	2.14	2.09	2.40[f]	.09
Ketones[h], uM	450	604	650	687	505	432	459	403	57
Growth hormone, ng/ml	9.52	9.74	10.07	9.04	9.71	8.68	9.52	9.14	.35
Insulin, ng/ml	1.07	.80[c]	.83	1.03	.92	.93	1.24	1.00	.10
Growth hormone/ insulin	10.3	14.8[b]	14.3	11.7	10.5	9.9	12.5	9.3	1.9

[a]contrast C vs. S, T, W; p<.05.

[b]contrast CI vs. SI, TI, WI; p<.05.

[c]contrast C vs. CI; p<.05.

[d]contrast S vs. SI; p<.05.

[f]contrast W vs. WI; p<.05.

[h]acetoacetate plus 3-hydroxybutyrate.

rate of fatty acid influx from the diet. Growth hormone has also been shown to increase NEFA concentration. There were dietary effects on plasma NEFA concentrations in this study. Groups fed T and W had significantly lower concentrations of NEFA than the C-fed group. Overall, NEFA concentrations of all groups except for S were lower than that of C. NEFA concentration of all groups except T started to increase from the fourth week of experiment (See Appendix, Figure 16). Interestingly, cows fed S had the highest NEFA during the first two weeks, possibly due to the elevated concentration of growth hormone in plasma during the same period (See Appendix, Figure 21).

There was considerable variation in blood ketones due to dietary treatments. Therefore, average blood ketones were similar for all groups. Glucose concentration in plasma was not influenced by diet; however, cows fed WI tended to have the highest plasma glucose concentration over the experimental period. Plasma calcium was not different due to dietary treatments. Plasma magnesium concentration was lowest for CI. As a result, concentrations for SI, TI, and WI were significantly higher. Isoacid addition to the fat supplemented diets increased mean plasma magnesium concentration, but the difference was significant only for diet W.

Nutrient partitionings toward the mammary gland or

adipose is responsive to hormonal control. Higher growth hormone in plasma stimulates partitioning of more nutrients toward the mammary gland for milk synthesis in early lactation. Groups fed CI and S had the highest milk yield and also highest plasma growth hormone concentration. However, differences in growth hormone concentations were not significant due to diets. Plasma growth hormone (See Appendix, Figure 21) tended to decrease over time (P=.054).

Insulin has been inversely associated with growth hormone. Insulin increases during lactation and partitions nutrients to adipose tissues (15, 52). The group fed W had lowest milk yield and highest plasma insulin. Conversely, groups fed S and CI had highest milk yield and lowest plasma insulin. When isoacid was added to diet C , it significantly reduced plasma insulin level. But milk production was not significantly increased. Insulin in plasma increased over time (See Appendix, Figure 22).

Growth hormone to insulin ratio has been related to milk yield. The CI and S groups had the highest milk yield and highest hormone ratio. The group fed W had the lowest milk yield and lowest ratio. However, the only significant difference due to dietary treatment occurred when isoacid was added. The hormone ratio was significantly higher in CI than in SI, TI, and WI.

RUMINAL VOLATILE FATTY ACIDS

Proportions and concentrations of ruminal volatile fatty acids are listed in Table 7. The C-fed group had the lowest concentration of all individual and total ruminal volatile fatty acids. Fat supplementations yielded significantly higher total and individual volatile fatty acids, except propionate. However, the ratio of acetate to propionate was not altered by fat supplementations, because propionate concentration increases (P=.06) were sufficient to maintain the ratio.

Isoacid additions to all diets produced higher isobutyrate and isovalerate concentrations in the rumen, possibly due to their presence in the isoacid mixture. Acetate and total volatile fatty acids were highest for groups fed CI and S. The CI-fed group had higher acetate, propionate, isobutyrate, butyrate, isovalerate, and total volatile fatty acids than the C-fed group. In general, concentrations of acetate, butyrate, and total volatile fatty acids were highest in cows fed CI and S. These groups had the highest milk production.

Table 7. Rumen volatile fatty acids (VFA) (uM/ml) associated with the feeding of control (C), calcium stearate (S), tallow (T), and whole whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

	C	CI	S	SI	T	TI	W	WI	SE
Total VFA	79[a]	110[b,c]	108	103	102	95	98	97	6
Acetate	47.7[a]	65.6[b,c]	65.8	62.0	62.1	54.8	57.6	57.0	3.3
Propionate	17.9	24.7[c]	21.6	21.4	21.6	22.3	23.1	23.7	2.4
Isobutyrate	.76[a]	1.46[c]	1.17	1.79[d]	1.01	1.41[e]	1.01	1.58[f]	.13[g]
Butyrate	9.5[a]	13.7[c]	14.7	13.3	13.2	12.0	11.4	10.5	1.2
Isovalerate	1.43[a]	2.12[c]	1.94	2.52[d]	1.79	2.03	1.96	2.35[f]	.20[g]
Valerate	1.37[a]	2.14	2.40	2.08	1.78	1.92	2.91	1.79[f]	.36
Acetate/ propionate	2.80	2.85	3.16	2.83	2.98	2.53	2.45	2.55	.31

[a] contrast C vs. S, T, W; $p<.05$.

[b] contrast CI vs. SI, TI, WI; $p<.05$.

[c] contrast C vs. CI; $p<.05$.

[d] contrast S vs. SI; $p<.05$.

[e] contrast T vs. TI; $p<.05$.

[f] contrast W vs. WI; $p<.05$.

[g] contrast C, S, T, W vs. CI, SI, TI, WI; $p<.05$.

DIGESTIBILITIES AND GLUCOSE AND EPINEPHRINE CHALLENGES

Apparent digestibility coefficients of dry matter, crude protein, and acid detergent fiber (Table 8) were not affected by dietary treatments. Glucose parameters (Table 9) for cows fed C, S, T, and W were not different. Normally glucose clearance rate is rapid in high-producing cows and highly dependent upon the quantity of dry matter intake. Variance associated with glucose clearance, space, and pool size was high, because only one injection was given to each of six cows per group.

Plasma NEFA parameters after epinephrine challenge were not altered by dietary treatments (Table 9). Epinephrine stimulates fatty acid mobilization from adipose tissues resulting in higher NEFA concentration in blood. The S-fed group had the highest plasma NEFA concentration above baseline and curve area, and lowest time to peak concentration. In general, plasma NEFA concentrations above baseline and areas of fat supplemented groups were greater than those of group C. Average time to peak concentration for all groups was 11 to 12 minutes after epinephrine injection.

Table 8. Apparent digestibility coefficients associated with the feeding of control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets and diets (CI, SI, TI, WI) with added isoacids.

	C	CI	S	SI	T	TI	W	WI	SE
	---------- % ----------								
Dry matter	77.4	70.3	73.9	75.5	76.8	69.9	72.4	69.5	3.7
Crude protein	70.2	73.1	73.8	71.2	71.5	72.6	66.4	65.4	3.6
Acid detergent fiber	55.3	49.9	48.7	49.7	54.7	53.1	50.0	49.5	4.1

Table 9. Glucose parameters, and responses in plasma non-esterified fatty acid (NEFA) to epinephrine challenges by control (C), calcium stearate (S), tallow (T), and whole cottonseed (W) diets for cows.

	C	S	T	W	SE
Glucose parameters					
Clearance rate, %/min[a]	7.71	7.34	8.37	7.84	.79
Pool space, l	58.2	73.4	57.0	61.8	5.7
Pool size, g	32.2	39.2	31.3	37.4	3.8
NEFA parameters					
Concentration, uEq/l[b]	52.1	62.8	52.7	52.4	4.7
Area	1,685	2,015	1,810	1,884	256
peak time, min.	11.2	10.6	12.0	11.8	.7

[a]After injection of .1 gm glucose/kg body weight.

[b]Average concentration above baseline after injection of epinephrine (.7 ug/kg body weight).

DISCUSSION

Normally, maximum dry matter intake enables cows to produce maximum milk. Previous studies have shown isoacid addition to dairy rations has resulted in unchanged or slightly reduced dry matter intake (7). In the present study, however, isoacid addition increased dry matter intake (Table 3, Figure 4). The group fed WI had the highest dry matter intake. Consequently, isoacid made W diet more acceptable and responsive.

Heinrichs et al. (49) suggested that fat supplementation should be avoided in time-limited feeding conditions, because cows tended to have a reduced length and increased number of meals. Fat supplementation may also reduce feed intake due to the negative effect of dietary free fatty acids on rumen digestion and its high caloric density (91). Usually, depressed digestion is accompanied by reduced feed intake (26). Dry matter intake tended to be reduced by a 5% bleachable tallow (94), was maintained by protected oils (10, 44) and whole cottonseed (1.9 kg per cow per day and up to 20%, respectively) (3, 33), and reduced with increasing dietary whole cottonseed (35% and 33%) (29, 48). In the present study, dry matter intake was not influenced by fat

supplementations.

Increased dry matter intake was not accompanied by a significant increase in milk yield (Table 3). However, CI- and S-fed groups produced 1.1 kg/day more milk than the C-fed group. According to Palmquist et al (91), a 5% increase in milk yield often was not statistically significant with less than 10 cows per group. In the previous study (51) by our laboratory, milk yield was significantly increased by calcium stearate. According to Heinrichs et al. (50), fat supplementations increased milk yield for high-producing cows. Thus, fat feeding must be more responsive to high producing cows limited in energy consumption. Milk yield response to W was low. Isoacid addition to W was favorable. Normally when isoacid was added to low quality or low protein diets (7, 38), milk yield responses were more positive probably due to improved microbial protein synthesis (7, 30). According to one report (97), isoacids absorbed from the digestive tract were transported toward body tissues rather than mammary gland tissues. Isoacid addition to C, S, and T diets was accompanied by decreased weight gain. Response to TI was most negative (Figure 6). Felix et al. (38) reported slightly reduced body weight gain by isoacid addition to a diet containing urea as a protein supplement. The weight gain response to WI was positive. For heifers, Felix et al.

(57) suggested that isoacid increased the growth rate of younger heifers due to more protein accumulation in the body.

It can be theorized that diets containing supplemental fat may favor weight gain, due to high caloric density. Previous research by our laboratory (51) observed increased weight gain in S and T diets. However, in the present study, net body weight gain was negative when fat supplemented diets were fed. The body weight loss for S and T diets may be attributed to the use of more energy for milk synthesis. For fat supplemented diets, body weight was increased during the last two weeks of experiment (Figure 6), and accompanied by decreased milk fat percent during the same period (See Appendix, Figure 8). Consequently, body weight gain may be negatively related to milk fat percent because weight gain and fat in milk are highly dependent upon the degree of fat mobilization from adipose tissues. Concerning seasonal differences in body weight gain, Miller et al. (84) reported weight gain in summer and weight loss in winter.

Efficiency of energy utilization was highest for C diet and lower for fat supplemented diets, primarily due to the low response to W. Those of other diets were not different from control. In a whole cottonseed feeding trial (1.9 kg per day per cow), Anderson et al (3), however, observed no difference in efficiency of energy utilization. Kronfeld et

al. (68) suggested that feeding a protected tallow for 14 weeks increased efficiency of utilization of metabolizable energy.

Fat content in milk depends on transfer of dietary long-chain fatty acids to milk, de novo synthesis in the mammary gland, and fat mobilization from adipose tissues stimulated by growth hormone (15, 91). Unprotected oils and fats tended to depress percent milk fat possibly due to disturbed ruminal digestion from toxic long-chain fatty acids (34, 93, 95, 122, 127). Protected lipids including calcium stearate, tended to increase percent milk fat (8, 10, 44, 51, 75, 95, 99, 113, 126). Whole cottonseed did not affect milk fat (3, 29, 48). In the present study, fat supplementations depressed milk fat possibly due to a relative increase in milk production (Table 4). Other sources of milk fat depression may be low fiber digestion caused by dietary fat (40, 91), depressed de novo synthesis of short-chain fatty acids in the mammary gland caused by dietary long-chain fatty acids (95, 112), decreased glucose uptake in the mammary gland (31), low efficiency of energy utilization (120), and undetected long chain fatty acids in milk fat using Infrared Analysis (39, 95). However, low fiber digestion was not evident in the present study as shown in Table 8. Iscacid addition restored milk fat percent to

normal or slightly above normal. It demonstrated that isoacid was more effective under these milk fat depressed conditions. Milk fat production was elevated by isoacid addition. This response was the result of increased milk fat percent. Response to W was obviously low (Table 5, Appendix, Figure 12) due to the lowest percent milk fat. Calculation of 4% fat-corrected milk was not the same as actual milk yield, but varied with fat content of milk. Isoacid addition to less responsive W diet showed the highest response. Responses to isoacid were similar to literature reports. Felix et al. (38) observed increased fat-corrected milk where isoacid was added to diets containing urea as a protein supplement.

Milk protein depends on the availability of glucose, amino acids, and propionate in the mammary gland. It has been suggested that fat supplementations resulted in milk protein depression caused by decreased casein in milk (33). However, all diets in this study except CI were similar in milk protein percent. Milk protein is relatively difficult to change by dietary manipulations. Protein yields were similar among diets. Milk lactose is negatively associated with milk fat and protein (35). Also, it depends on the availability of glucose from blood (71). Lactose was affected by diets in this study. Isoacid reversed lactose

depression caused by T and W diets. Increased dry matter intake enables cows to utilize more dietary glucose for lactose synthesis. This icreased dry matter intake by isoacid addition may be attributed to the recovery of depressed lactose. Lactose yields also were not different except for significantly low response to W. Similar responses of milk component yields to diets indicated that differences in percent components may be attributed primarily to increased milk yield.

Somatic cell counts were not different among diets due to great variations. Raubertas et al. (101) suggested that somatic cell counts can be used as a genetic index for resistence to mastitis.

Concentrations of metabolites in jugular blood are related to nutritional status of lactating cows. Metabolites can be affected by nondietary factors, such as herd, stage of lactation, season, and performance of cows (59). Seasonal factors should also be considered in long-term experiments. Dietary treatments influenced levels of non-esterified fatty acids, ketones, magnesium, and insulin concentrations in jugular blood. In early lactation, blood non-esterified fatty acids increase with adipose fat mobilization stimulated by growth hormone (123). High energy demand in early lactation requires nutrient partitioning from adipose to the

mammary gland (27). Plasma non-esterified fatty acids were low for T and W diets. Isoacid addition to T and W elevated the low concentrations. Generally, results were not consistent. These inconsistent responses were similar to literature reports. Responses to protected lipids were usually positive (44, 102, 110), but responses to unprotected oils were either positive (44, 122), negative (44), or unaltered (93). Plasma non-esterified fatty acids were highest for diet S, which showed the highest growth hormone in plasma. Blood glucose is inversely associated with glucose demand for milk synthesis (52). Lee et al (69) warned that blood glucose should be used carefully in indicating insufficient energy because glucose concentrations in plasma may be affected by other non-dietary factors. Plasma glucose was not affected by dietary treatments.

Blood ketones are inversely associated with plasma glucose (46, 55). In early lactation, plasma glucose decreases, and blood ketones increase because inadequate glucose for milk synthesis stimulates fat mobilization from body reserves resulting in ketogenesis (55). Blood ketones in this study were affected by diets. However, there was no significant difference in orthogonal contrast due to great variations. According to Hove (55), blood ketone levels are usually variable in healthy cows.

Nutrient partitioning for milk synthesis in early lactation occurs under hormonal control (15, 27), which seems to be highly related to performance of lactating cows. According to Hart et al (47), the level of plasma growth hormone was positively associated with milk yield and plasma non-esterified fatty acid levels and negatively asscciated with body weight gain. Insulin was positively correlated with body weight gain. Growth hormone to insulin ratio was positively related to milk yield. In the present study, growth hormone was not altered by dietary manipulations. However, responses were similar to those of Hart (47). Plasma growth hormone was relatively high for CI and S which were highest in milk yield, relatively high in non-esterified fatty acids, and lowest in net weight gain (excluding S) (Table 3 and 6). Change in plasma growth hormone for S was characterized by steep increase during the first week followed by a decrease during the second week of experiment (See Appendix, Figure 21).

Plasma insulin was reduced by isoacid addition to C. Insulin was relatively low for CI and S, which were highest in milk yield. Insulin was highest for W, which was lowest in milk yield (Table 3 and 6). Responses were not consistent with associated body weight gain. Growth hormone to insulin ratio response was not significantly influenced by

isoacid addition to fat diets, but within diets with isoacids added, CI was significantly higher than SI, TI, and WI (Table 6). Also, SI, TI, and WI tended to be lower than those of S, T, and W. With respect to time, growth hormone and growthe hormone to insulin ratio (52) increased and insulin decreased in early lactation, especially before 50 days (52, 123). In the present study, growth hormone (P=0.054) and growth hormone to insulin ratio decreased and insulin increased from the initiation of experiment (See Appendix, Figure 21, 22, and 23).

Considerable variation is typical for plasma calcium (77). Plasma calcium depends on dietary calcium and calcium mobilization from bone and kidney (27). Dietary treatments did not affect plasma calcium. This result was similar to that of other studies (51, 68). Lee et al. (69) reported that plasma calcium was influenced more by stage of lactation rather than dietary intake. Plasma magnesium was affected by diets. Response was lowest for CI and highest for WI (See Appendix, Figure 19). Consequently, plasma magnesium may be more easily altered by diets than plasma calcium. McAdam et al. (77) observed an increase in plasma magnesium due to trace mineral salt addition.

Dietary treatments may affect yields and proportions of volatile fatty acids and pH in the rumen (60, 124). Volatile

fatty acids produced by bacterial metabolism may be used for the synthesis of long-chain fatty acids in the rumen (23) or may be transported through the blood circulation for later use as energy sources (60). Statistical analyses (Table 7) revealed that isoacid addition to fat diets resulted in reduced ruminal acetate and total volatile fatty acid concentrations compared to CI. The results suggested that isoacid addition to fat diets inhibited acetate production, resulting in reduced total volatile fatty acid yields. The mechanism of this response is unexplained. Isoacid increased concentrations of acetate, propionate, isobutyrate, butyrate, and isovalerate, and total volatile fatty acids when added to C. The response may be due to stimulated rumen fermentation by isoacid. Isoacid increased isobutyrate in SI, TI, and WI and isovalerate in SI and WI, and decreased valerate in WI. The increased isobutyrate and isovalerate in all isoacid diets were primarily due to their presence in the isoacid mixture. In contrast, valerate responses to isoacid were not additive. Consequently, it is suggested that valerate may be more reactive in the rumen than any other components of isoacid.

Fat supplementations resulted in increased concentrations of individual volatile fatty acids, except propionate ($P=0.06$), and total volatile fatty acids. This response may

be due to conversion of more carbohydrate into volatile fatty acids or to degradation of dietary long-chain fatty acids during ruminal fermentation. However, no data was available to clearly explain the increased volatile fatty acids observed in cows fed fat supplemented rations. Acetate to propionate ratio was not significantly influenced by dietary treatments. However, the ratio was highest for S. The response to S was similar to that reported by Herbein (51). Overall, A/P ratio responses were inconsistent with milk yield responses. As in Jones' study (60), the estimated total metabolizable energy may be more responsible for milk yield change than ruminal acetate or propionate contents.

Branched-chain volatile fatty acids (isoacid) may stimulate cellulose digestion in the rumen (30) because branched-chain fatty acids are required for growth of cellulolytic bacteria (32). In normal diets, the major source of branched-chain carbon skeletons for the microbial growth is dietary protein and endogenous branched-chain amino acids (30). By adding isoacids to dairy rations, it would be expected that fiber digestion may be increased. However, in the present study, isoacid additions did not alter levels of digestibilities. Cummins et al. (30) suggested that high-protein diets may be less responsive to isoacid addition. Palmquist (91) has hypothesized that tallow-like

fats may inhibit cellulolytic bacterial growth in the rumen resulting in the depressed fiber digestion. He has further suggested that protected fats escaping rumen fermentation may prevent the unfavorable effect observed with unprotected fats. However, in our study, digestibilities of dry matter, crude protein, and acid detergent fiber were not influenced by dietary treatments. Digestibilities of dry matter and acid detergent fiber were highest for C in the present study, and digestibility of crude protein was highest for S. Results of S, T, and W rations on digestibilities are supported by those of Jenkins et al. (58), Palmquist et al. (94), and Coppock et al. (29), respectively. Coppock et al. (29) reported that a 30% whole cottonseed feeding increased digestibility of crude protein. However, this pattern of crude protein response was not observed in the present study. DePeters et al. (34) suggested that digestibilities tended to decrease as the level of dietary fats increased. In conclusion, the mechanism by which fat supplementations or isoacid additions to fat diets affect rumen fermentation is still not clear.

Glucose is more rapidly utilized for milk synthesis in early lactation than in late lactation. Fat supplementations may alter glucose metabolism, because fat increases the energy density of the diet. However, glucose clearance

rate, pool space, and pool size were not significantly different due to dietary treatments. Gebhart (41) reported the requirement of at least 12 experimental cows to detect significant differences in plasma glucose parameters. Glucose clearance rate represents the ability of the cows to utilize glucose. Herbein et al. (53) reported that glucose turnover was linearly related to digestible energy intake. Gebhart (41) observed a positive correlation between glucose clearance and dry matter intake. However, in the present study, these significant relationships were not apparent. In general, S and W diets caused lower clearance rates than C. This trend was comparable to the observations of Palmquist et al. (92) that protected fat feeding resulted in slower glucose clearance. In contrast, unprotected fat (tallow) had higher clearance than C. It might be suggested that glucose clearance was increased by unprotected fats and decreased by protected fats. Although glucose pool space and pool size were not different among diets in our study, consistent patterns were observed as follows; pool space and pool size were higher in S and W, and lower in T than in C. Consequently, lower glucose utilization resulted in higher pool space and size. Likewise, pool space and pool size were highest in S-fed group which consumed the most dry matter (Table 3). This was in agreement with the findings

of Gebhart (41) and Buckley et al. (22) that glucose pool size depended on dry matter intake. Additionally, pool space varied positively with pool size.

The mean values of non-esterified fatty acid (NEFA) parameters after epinephrine challenge are presented in Table 9. NEFA parameters were not significantly different due to dietary treatments. The greatest increase in NEFA above baseline in this study was in the group fed S. The S-fed group with highest milk production had the greatest ability to mobilize fats from adipose when stimulated by epinephrine injection. According to Bauman et al.(14) and Jaster et al. (56), fat mobilization is highly related to the population of beta-adrenergic receptors on the membrane surface of adipose tissues. Epinephrine response curve area was also highest for S. Overall, concentrations of plasma NEFA and area were also higher for groups fed fat supplemented rations than C.

SUMMARY AND CONCLUSIONS

Differences in milk yield were not significant, but CI, S, and WI averages tended to be higher than those of other diets evaluated in this study. CI had highest plasma growth hormone to insulin ratio and ruminal total volatile fatty acids, and lowest plasma insulin favoring milk production. Cows fed S had highest plasma non-esterified fatty acids, plasma growth hormone, and acetate to propionate ratio in the rumen. Cows fed WI had highest dry matter intake and fat-corrected milk. While fat supplementations raised dietary energy density, they resulted in depressed percent milk components and net body weight loss. In contrast, Isoacid addition led to increased dry matter intake, percent milk components, fat production, fat-corrected milk, and ruminal branched-chain volatile fatty acids over unsupplemented diets. Both fat supplement and isoacid addition maintained normal digestibilities of dry matter, crude protein, and acid detergent fiber. Cows fed W rations containing highest energy density, consumed lowest dry matter intake and responded less favorably than cows fed other diets. As a result, dry matter intake was a more important factor than energy density in improving performance of cows. To increase

energy density with fat supplements and simultaneously maximize dry matter intake, isoacid addition was favorable. Further studies should be conducted to investigate methods to improve dry matter intake of cows fed fat supplemented diets.

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APPENDIX

Table 10. Significant differences of contrasts analyzed by Bonferroni F test.

Variables	Significant contrasts
Dry matter intake	g
Weight gain	a, c
Milk fat, %	d, g
Milk lactose, %	a, e, f
Milk SNF, %	a, c, e
Milk fat yield	f, g
Milk lactose yield	f
Plasma NEFA	d
Plasma magnesium	b
Ruminal total VFA	a, c
Acetate	a, c
Isobutyrate	c, d, f
Butyrate	a, c
Isovalerate	c
Valerate	a, f

a: contrast C vs S, T, W; $p<.05$.
b: contrast CI vs SI, TI, WI; $p<.05$.
c: contrast C vs CI; $p<.05$.
d: contrast S vs SI; $p<.05$.
e: contrast T vs TI; $p<.05$.
f: contrast W vs WI; $p<.05$.
g: contrast C, S, T, W vs CI, SI, TI, WI; $p<.05$.

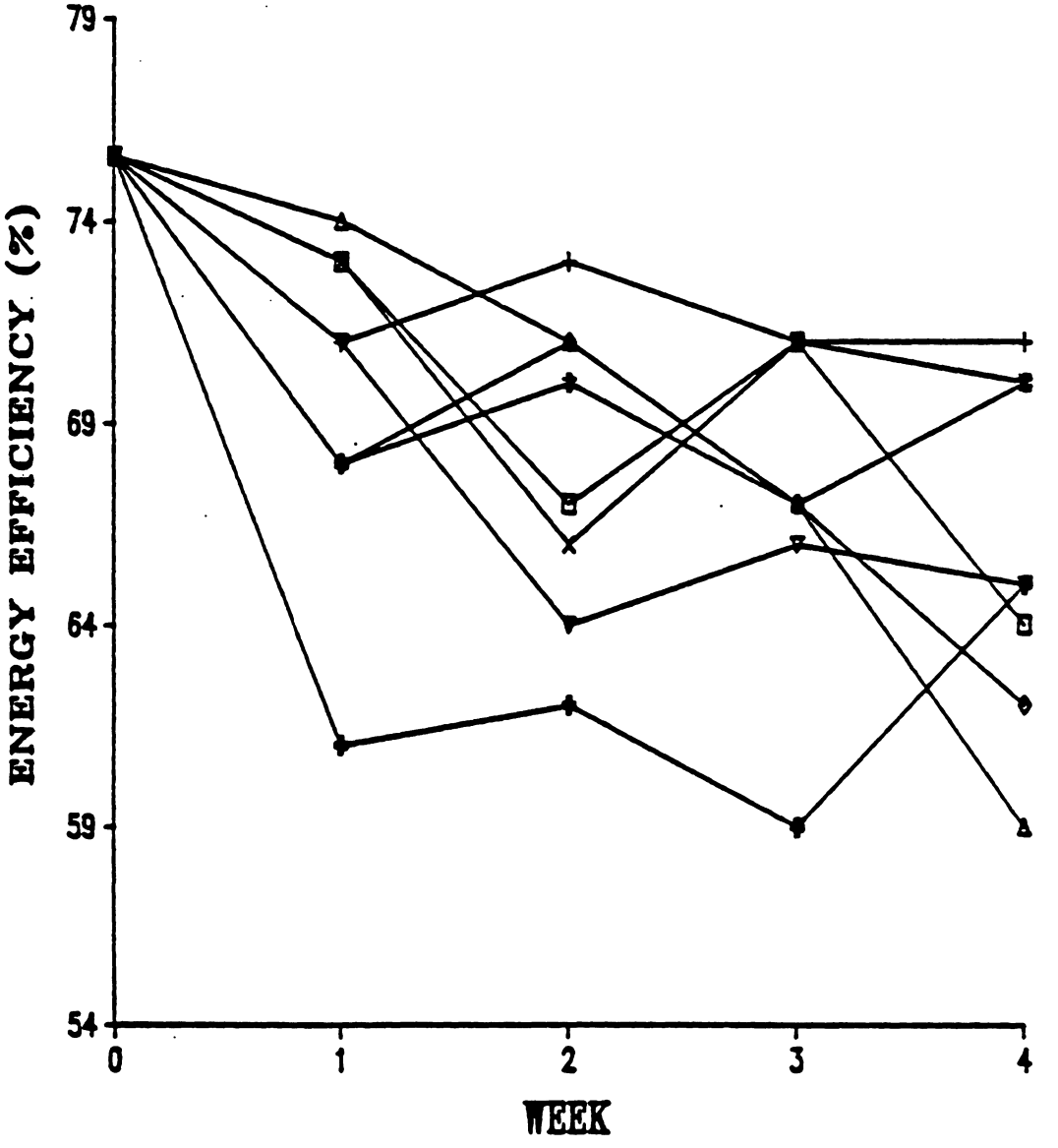

Figure 7. Efficiency of energy utilization by cows fed control (+), calcium stearate (X), tallow ($), and whole cottonseed (⌀) diets, and control (□), calcium stearate (Δ), tallow (◊), and whole cottonseed (▽) diets with added isoacids.

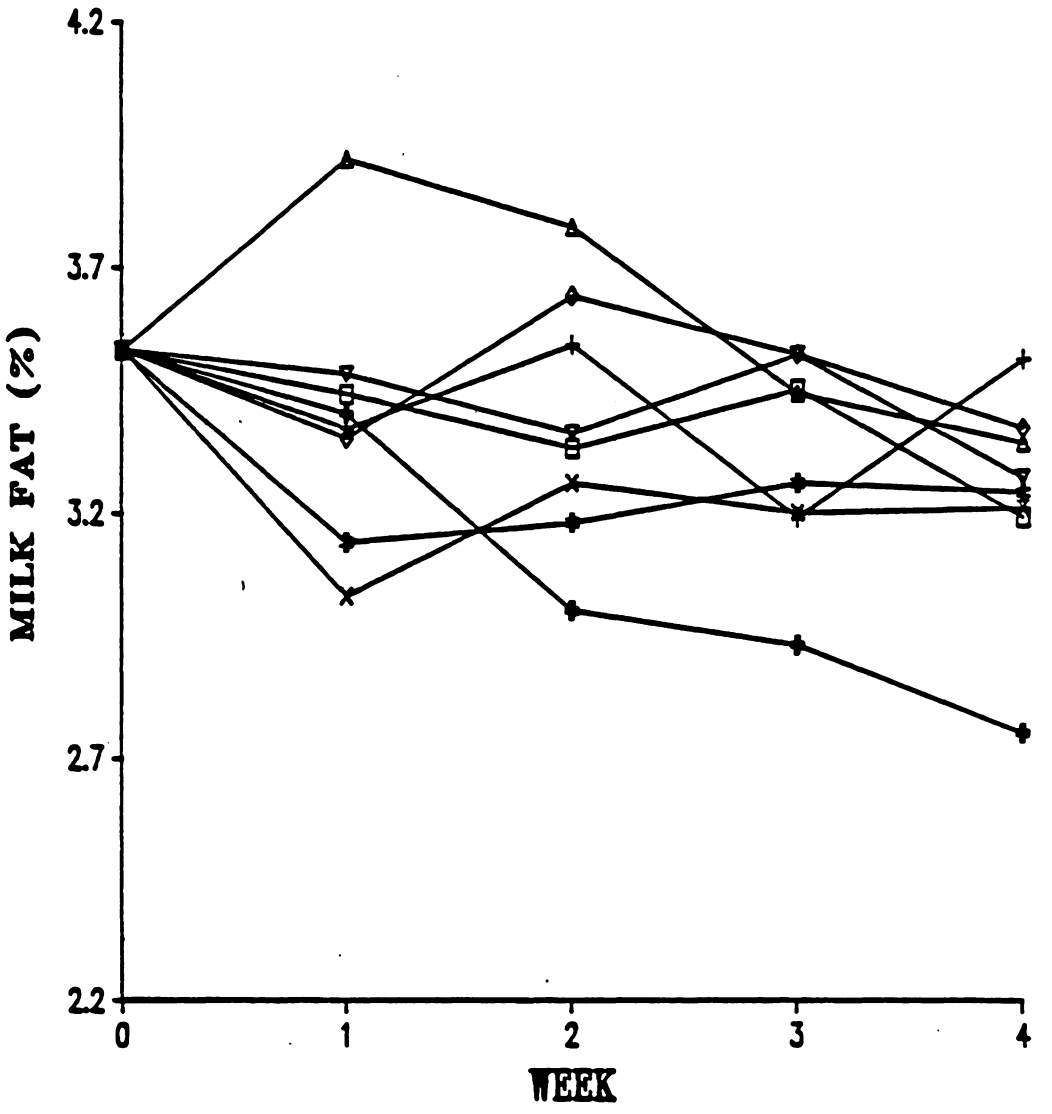

Figure 8. Percent milk fat of cows fed control (+), calcium stearate (×), tallow (✱), and whole cottonseed (✠) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

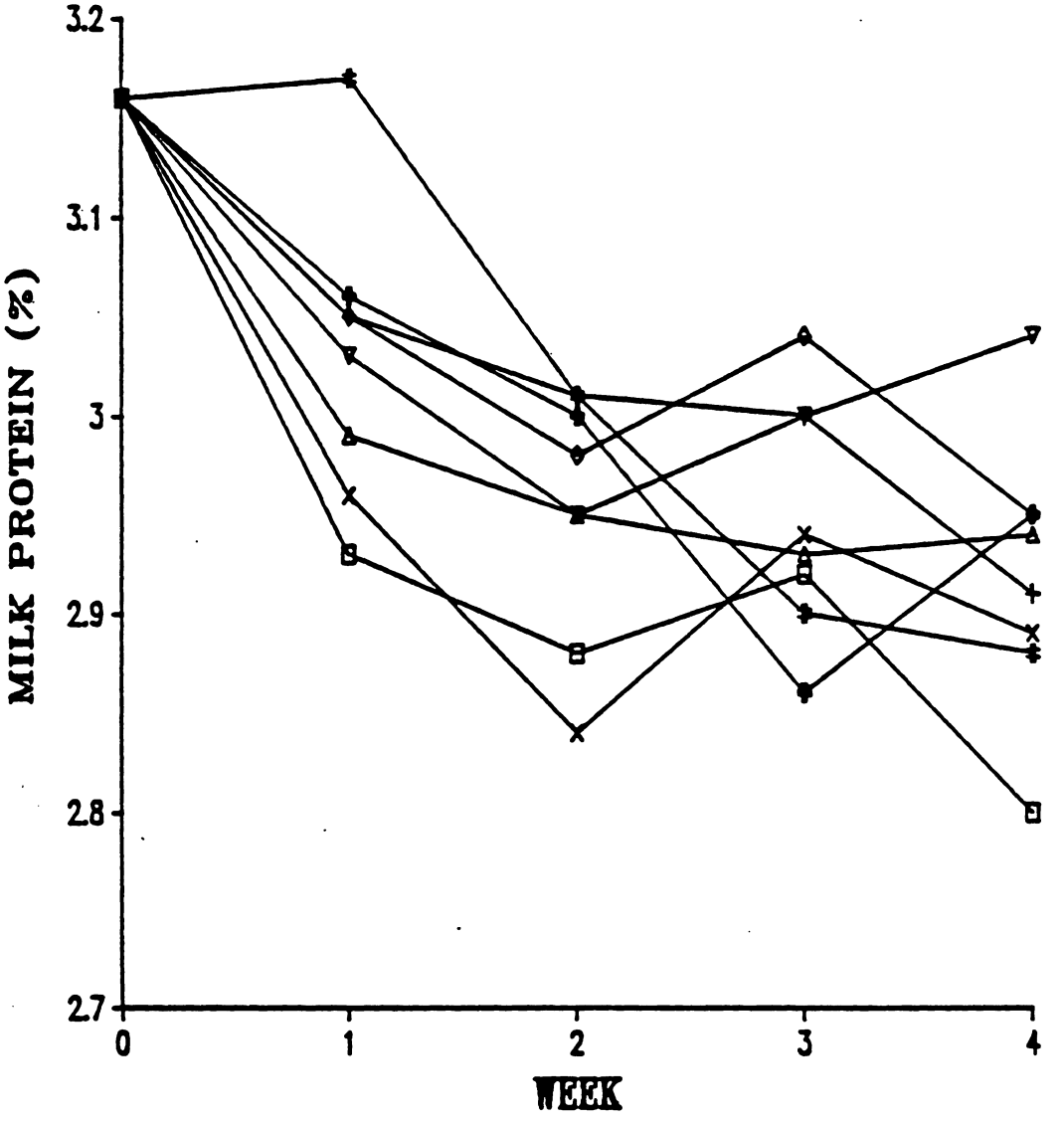

Figure 9. Percent milk protein of cows fed control (+), calcium stearate (X), tallow (‡), and whole cottonseed (⊕) diets, and control (□), calcium stearate (Δ), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

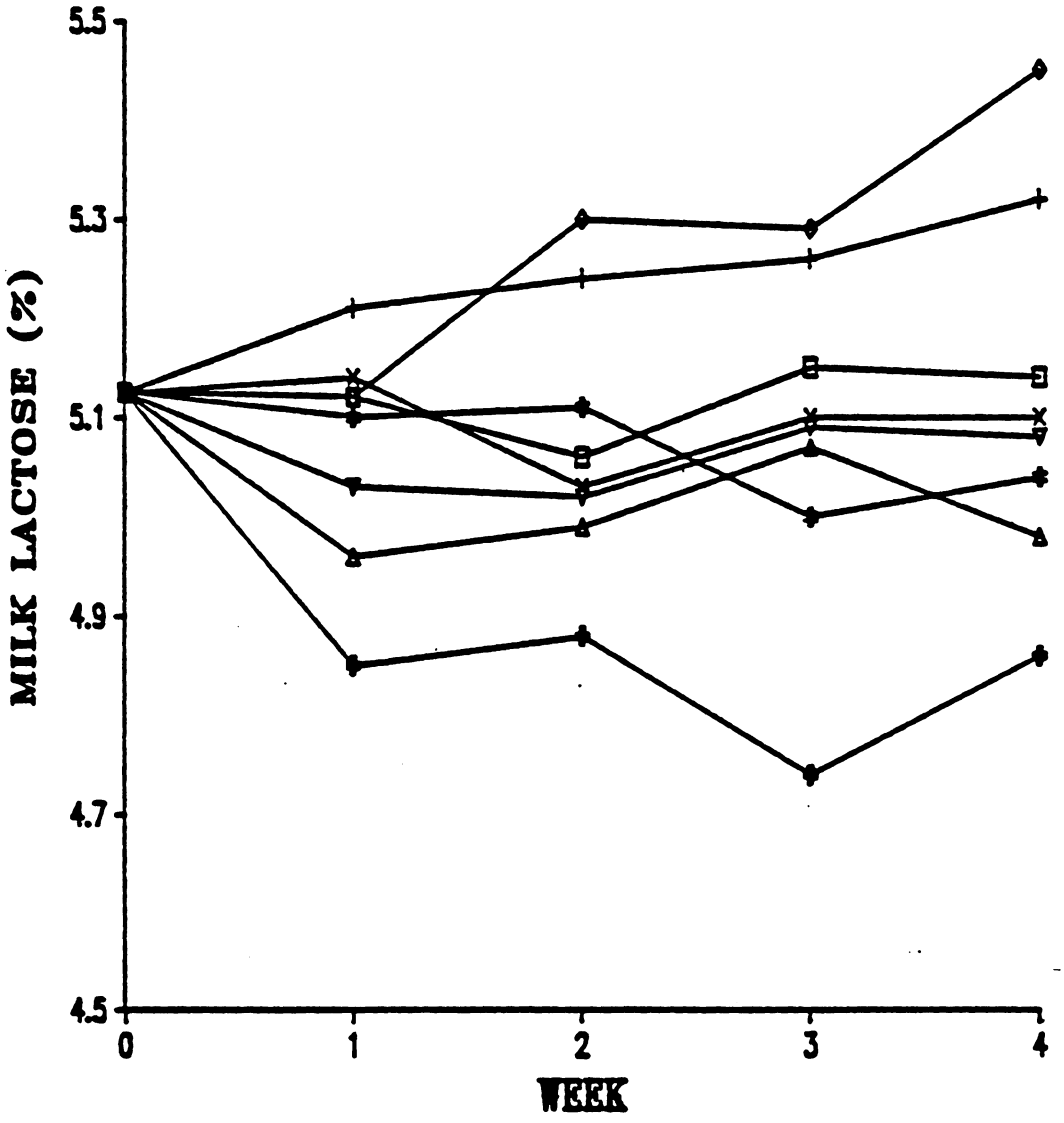

Figure 10. Percent milk lactose of cows fed control (+), calcium stearate (×), tallow (*), and whole cottonseed (⊕) diets and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

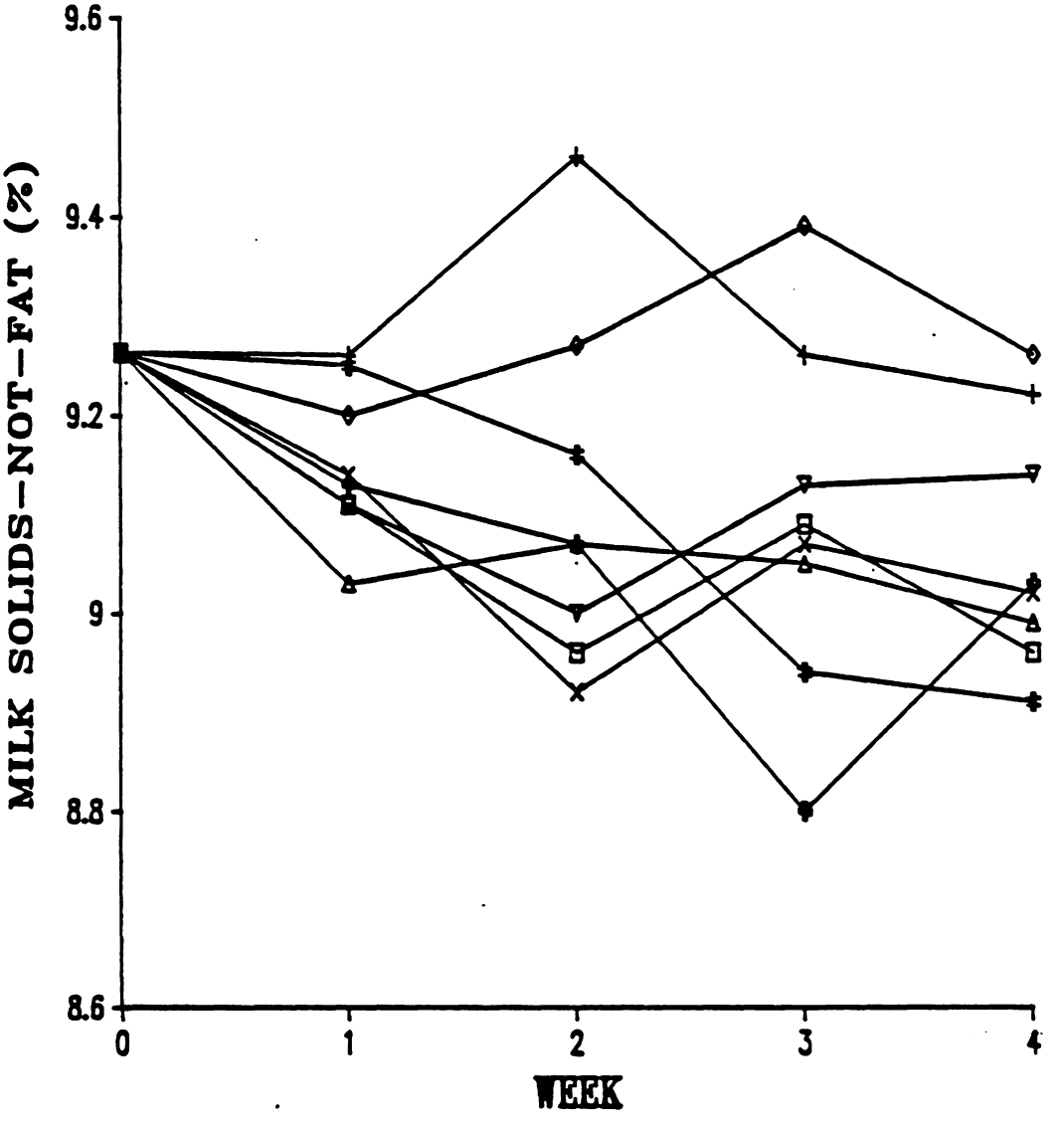

Figure 11. Percent milk solids-not-fat of cows fed control (+), calcium stearate (X), tallow ($), and whole cottonseed (Φ) diets, and control (□), calcium stearate (Δ), tallow (◊), and whole cottonseed (∇) diets with added isoacids.

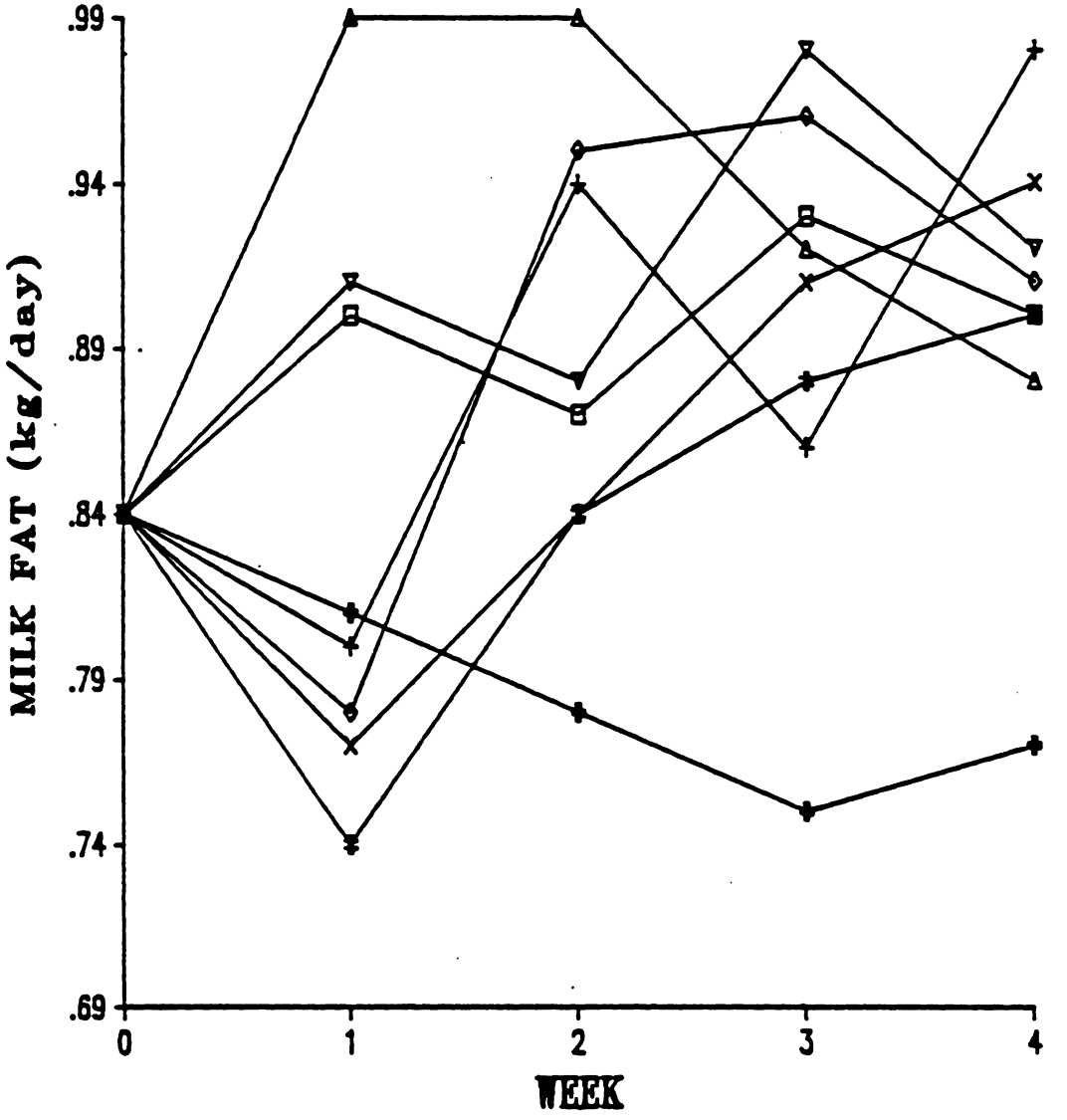

Figure 12. Milk fat yield of cows fed control (+), calcium stearate (X), tallow ($), and whole cottonseed (#) diets, and control (□), calcium stearate (△), tallow (◊), and whole cottonseed (▽) diets with added isoacids.

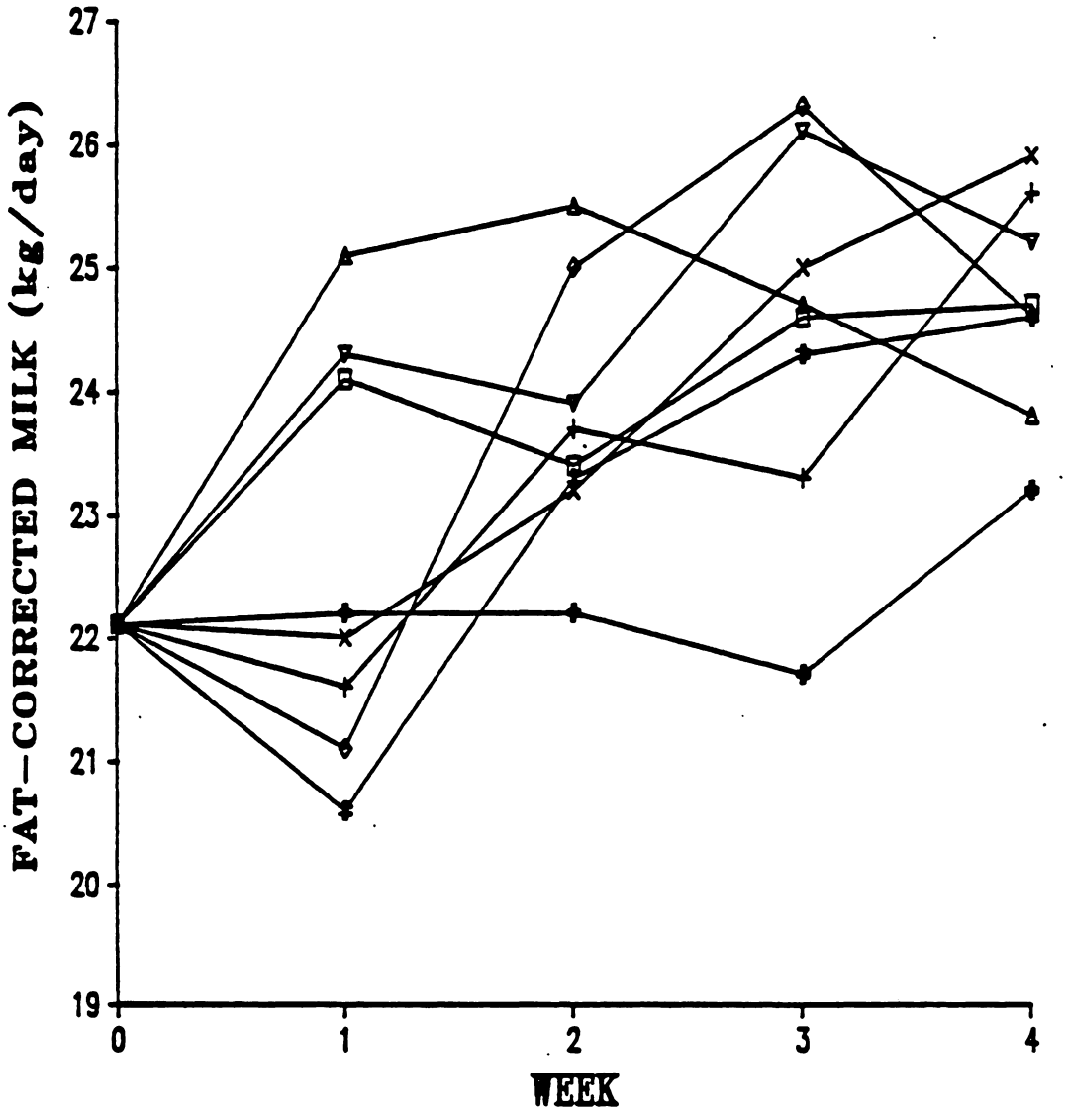

Figure 13. Fat-corrected milk yield of cows fed control (+), calcium stearate (X), tallow (*), and whole cottonseed (⊕) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

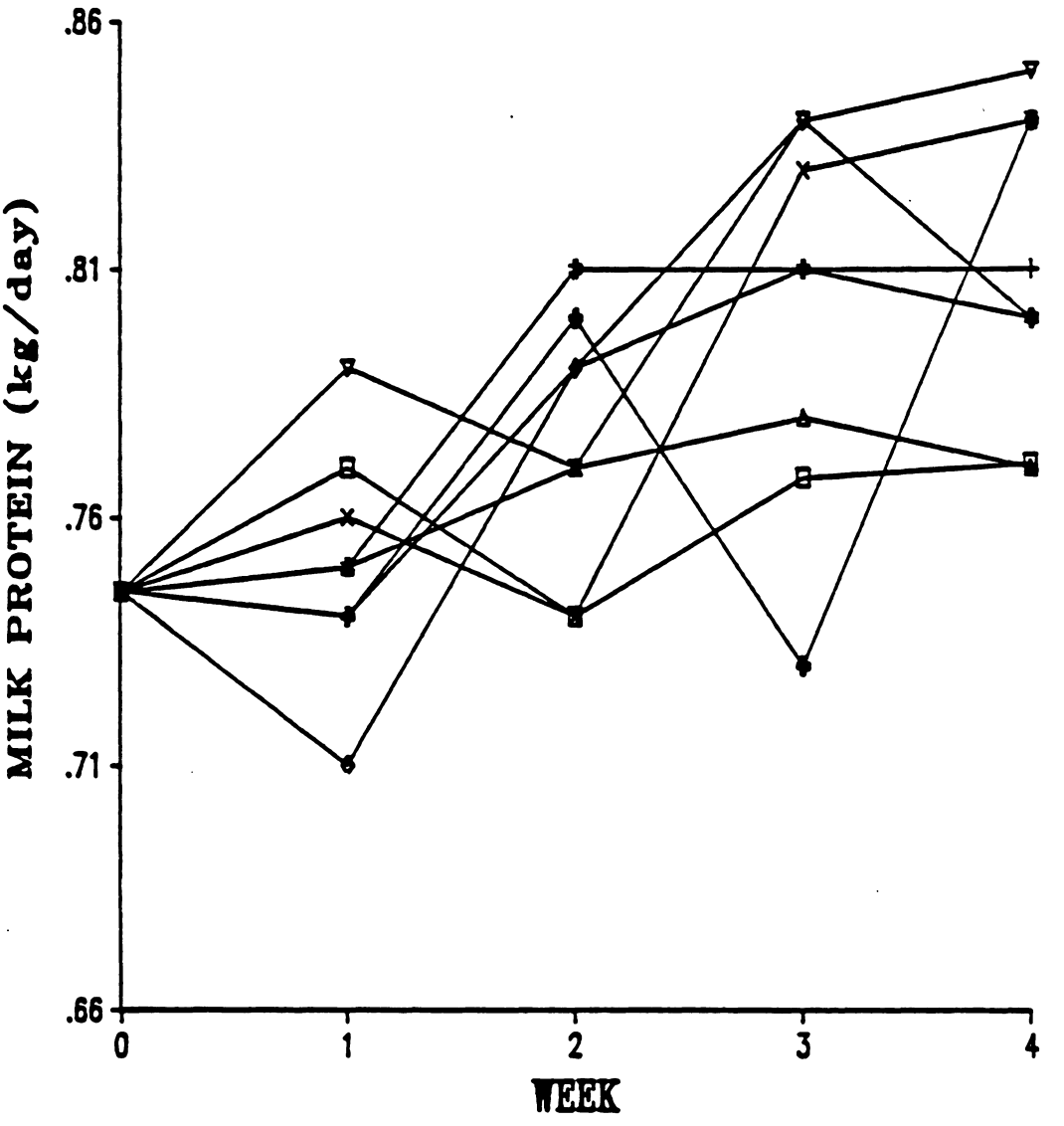

Figure 14. Milk protein yield of cows fed control (+), calcium stearate (X), tallow (‡), and whole cottonseed (Φ) diets, and control (□), calcium stearate (Δ), tallow (◊), and whole cottonseed (▽) diets with added isoacids.

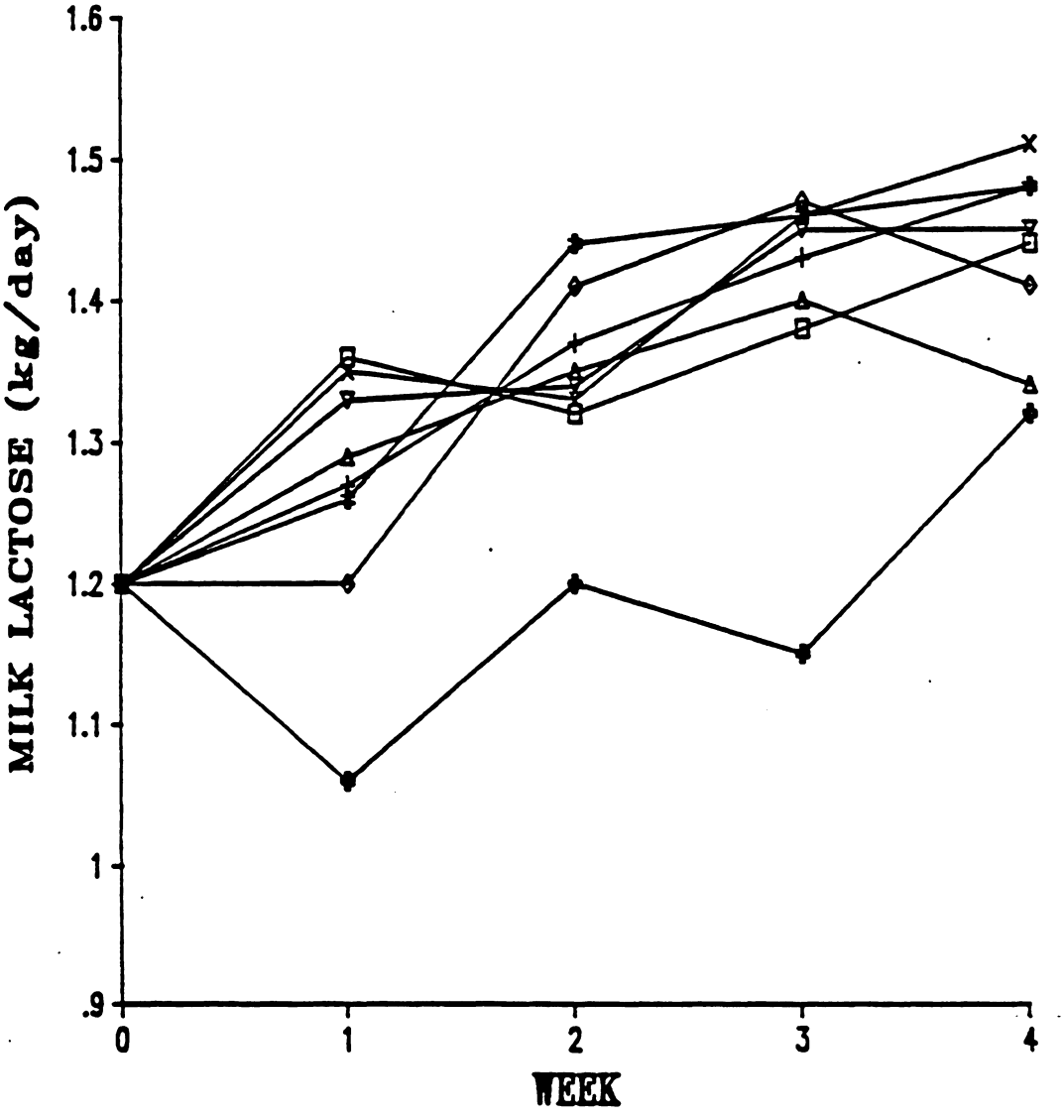

Figure 15. Milk lactose yield of cows fed control (+), calcium stearate (X), tallow (#), and whole cottonseed (⊕) diets, and control (□), calcium stearate (Δ), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

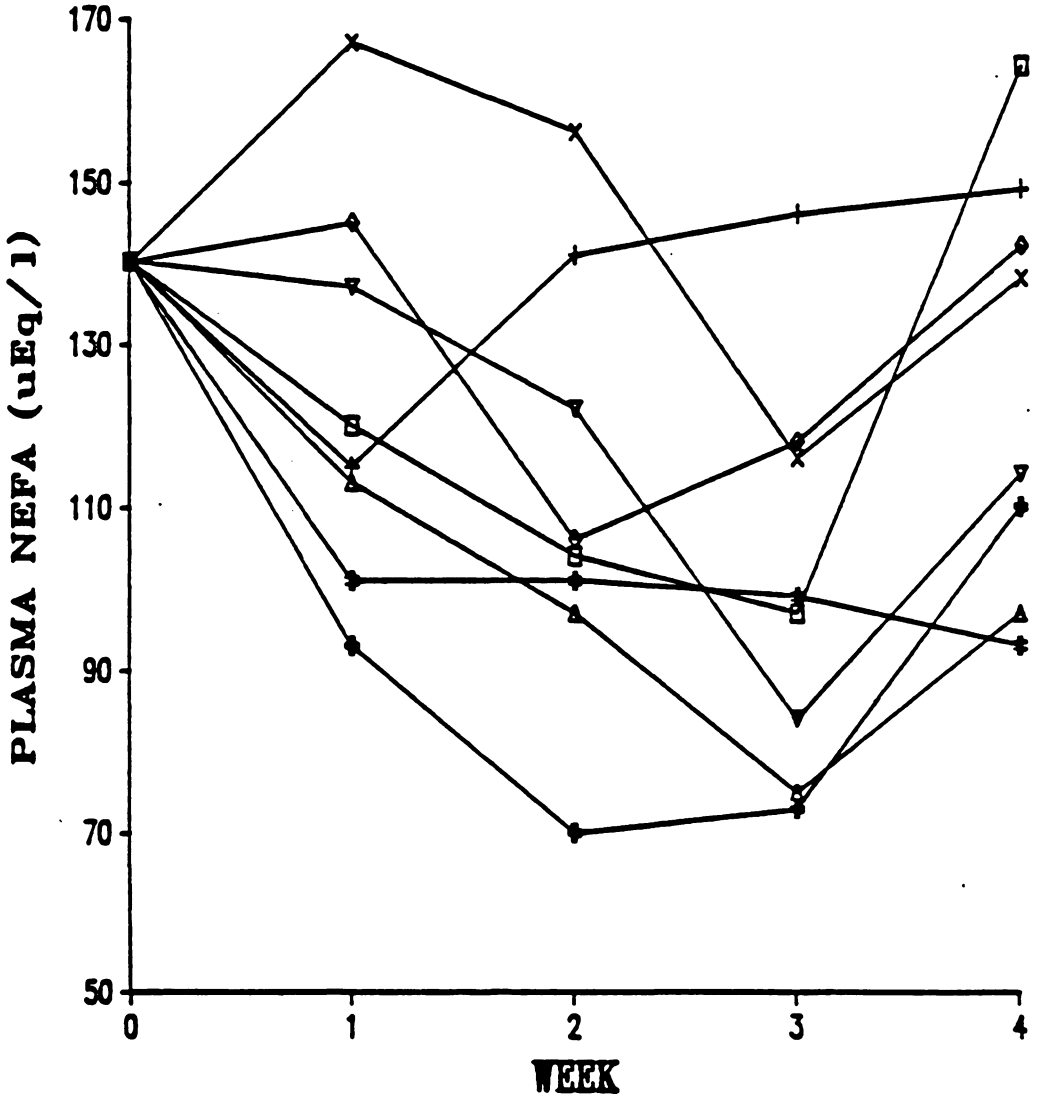

Figure 16. Plasma non-esterified fatty acid concentrations of cows fed control (+), calcium stearate (X), tallow (*), and whole cottonseed (⊕) diets, and control (□), calcium stearate (Δ), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

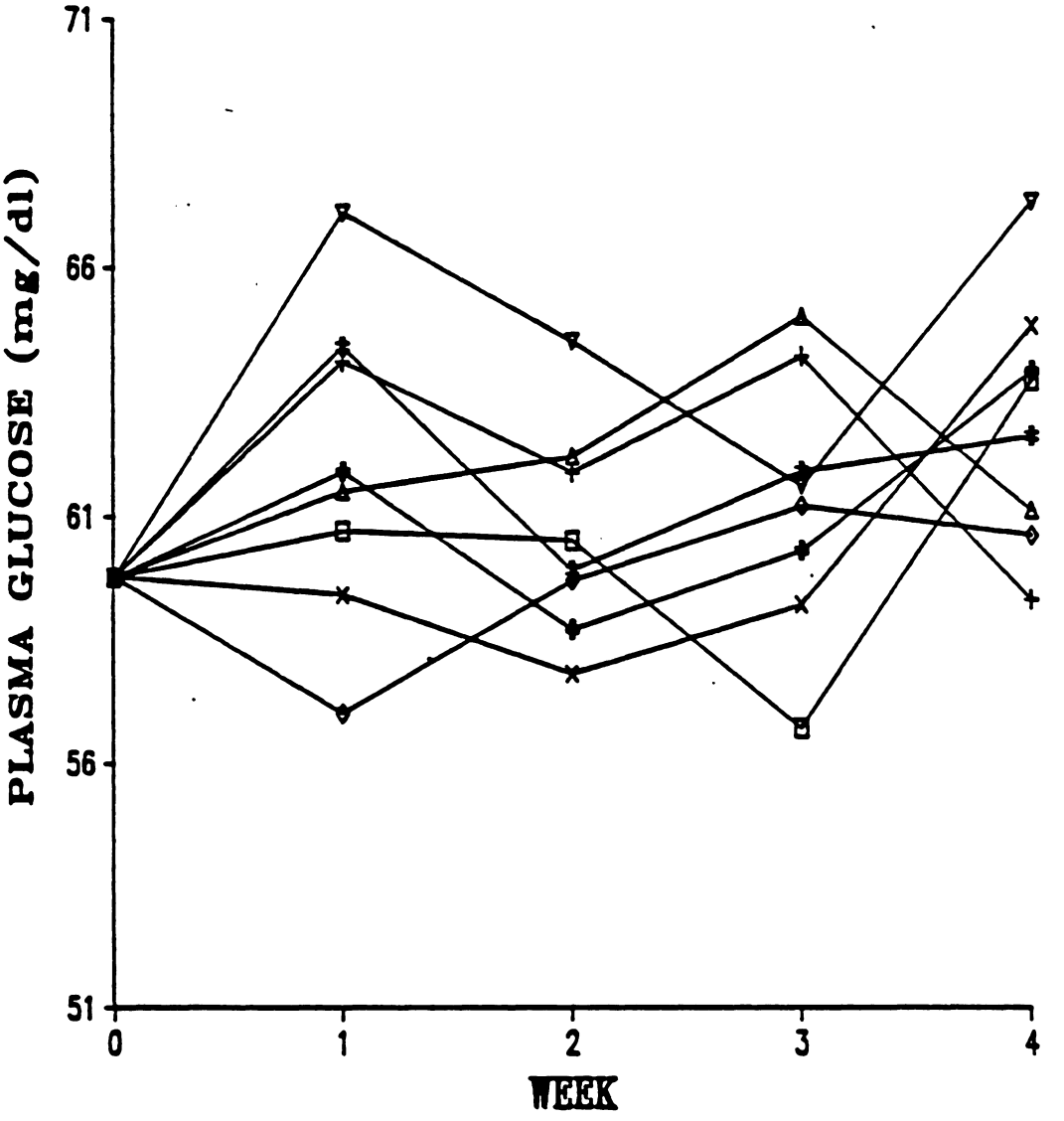

Figure 17. Plasma glucose concentrations of cows fed control (+), calcium stearate (X), tallow ($), and whole cottonseed (⊕) diets, and control (□), calcium stearate (Δ), tallow (◊), and whole cottonseed (▽) diets with added iscacids.

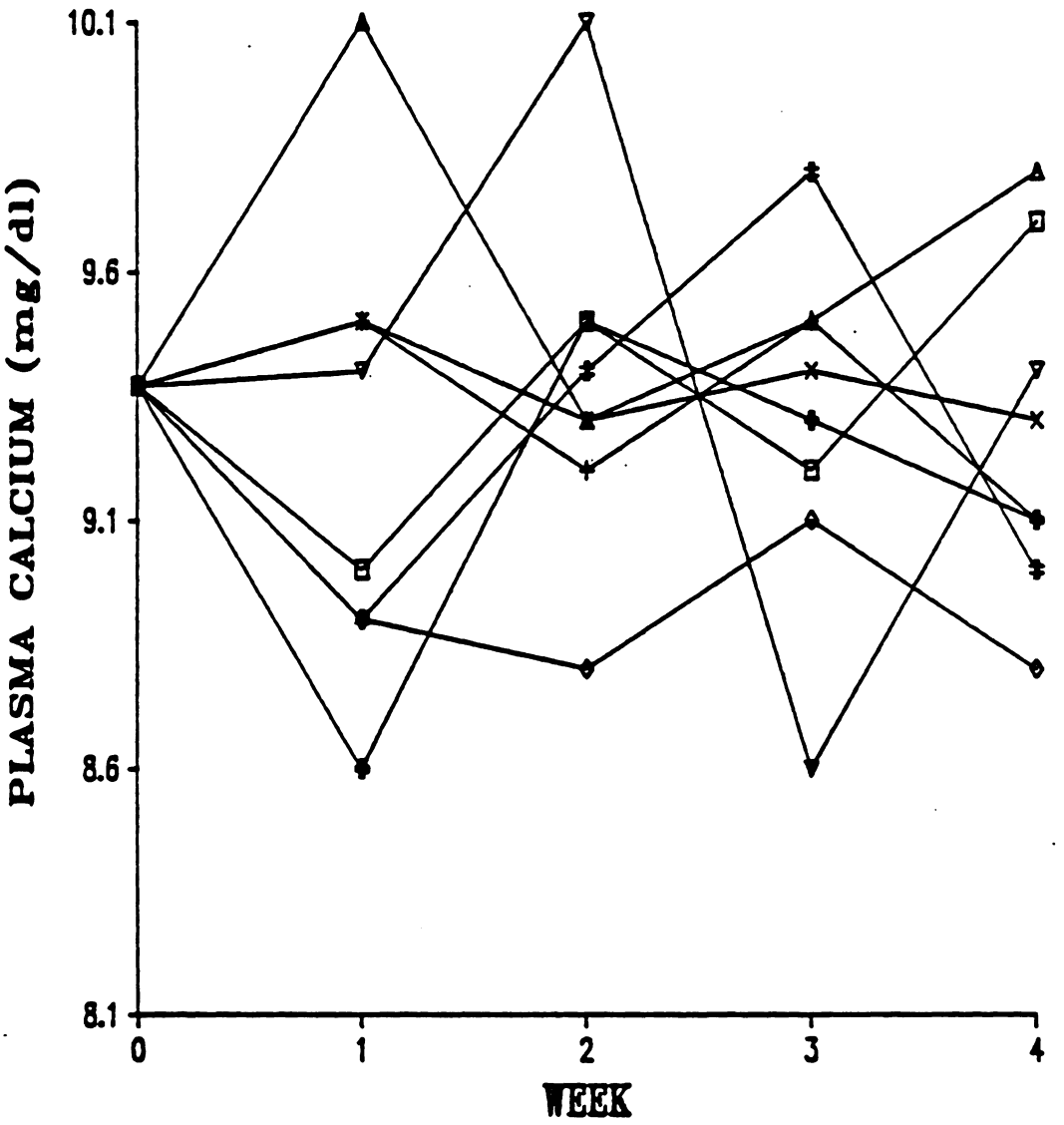

Figure 18. Plasma calcium concentrations of cows fed control (+), calcium stearate (X), tallow (*), and whole cottonseed (✣) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

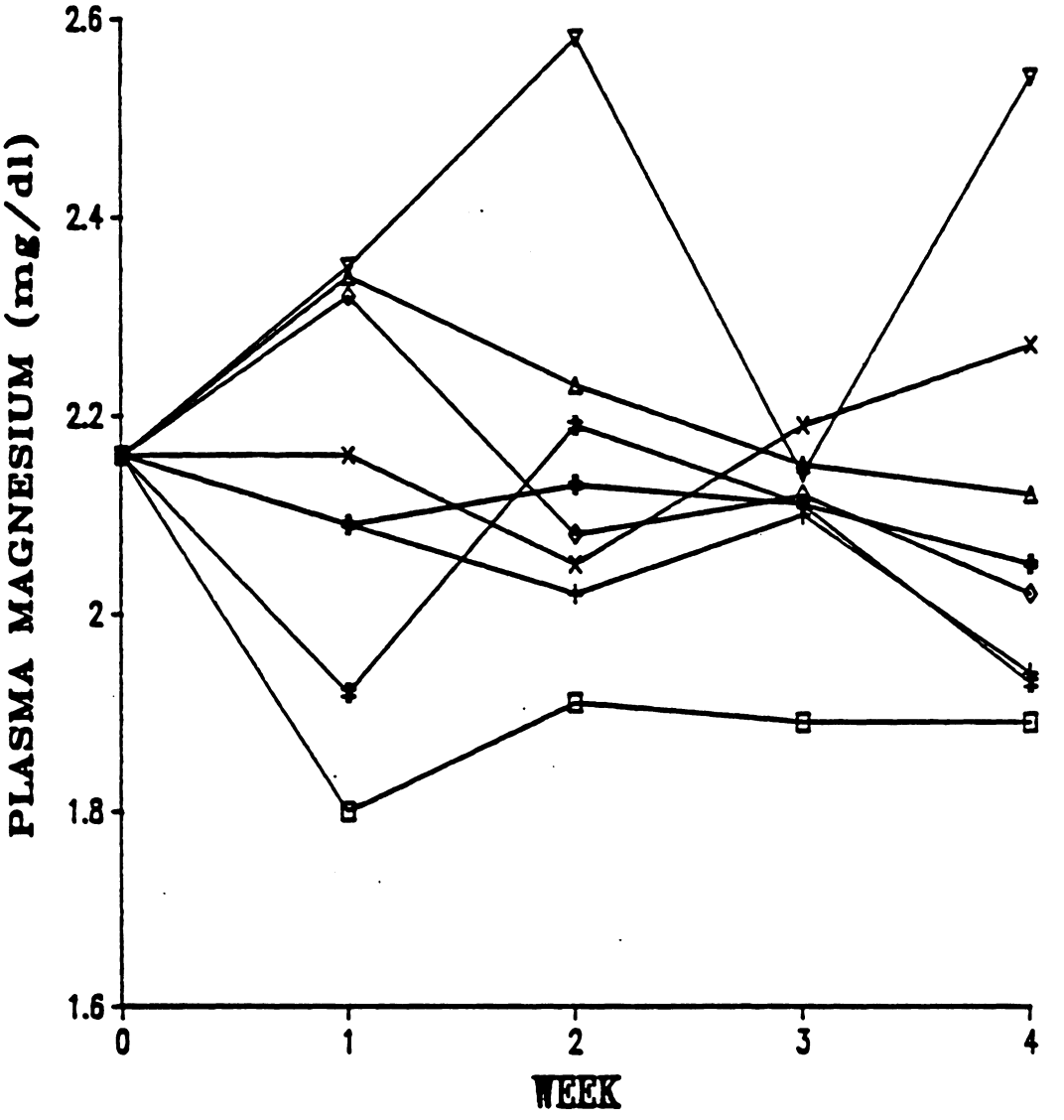

Figure 19. Plasma magnesium concentrations of cows fed control (+), calcium stearate (X), tallow ($), and whole cottonseed (✣) diets, and control (□), calcium stearate (△), tallow (◊), and whole cottonseed (▽) diets with added isoacids.

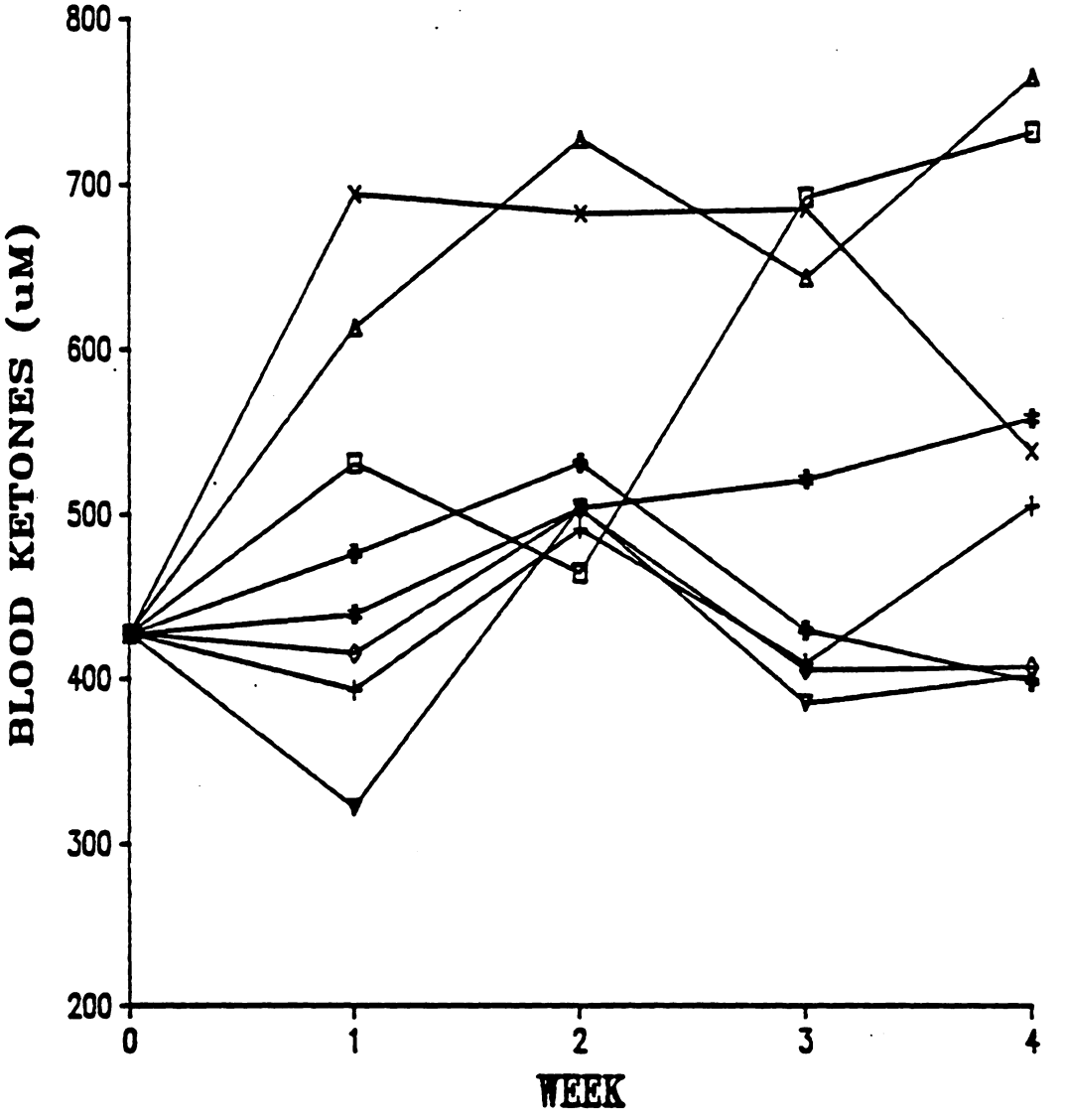

Figure 20. Blood ketone concentrations of cows fed control (+), calcium stearate (×), tallow (Ⅱ), and whole cottonseed (⊕) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

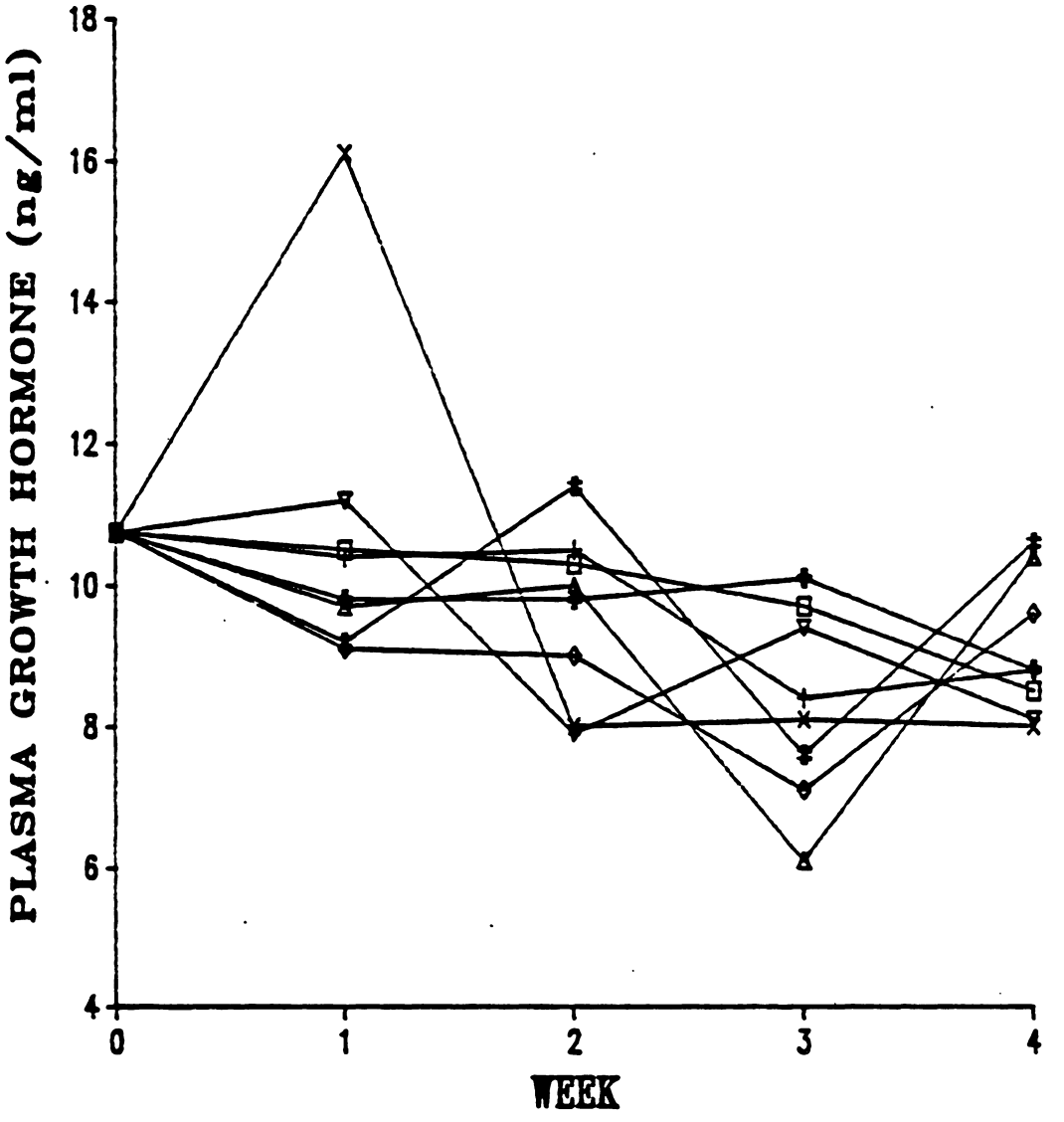

Figure 21. Plasma growth hormone concentrations of cows fed control (+), calcium stearate (×), tallow (✱), and whole cottonseed (✤) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

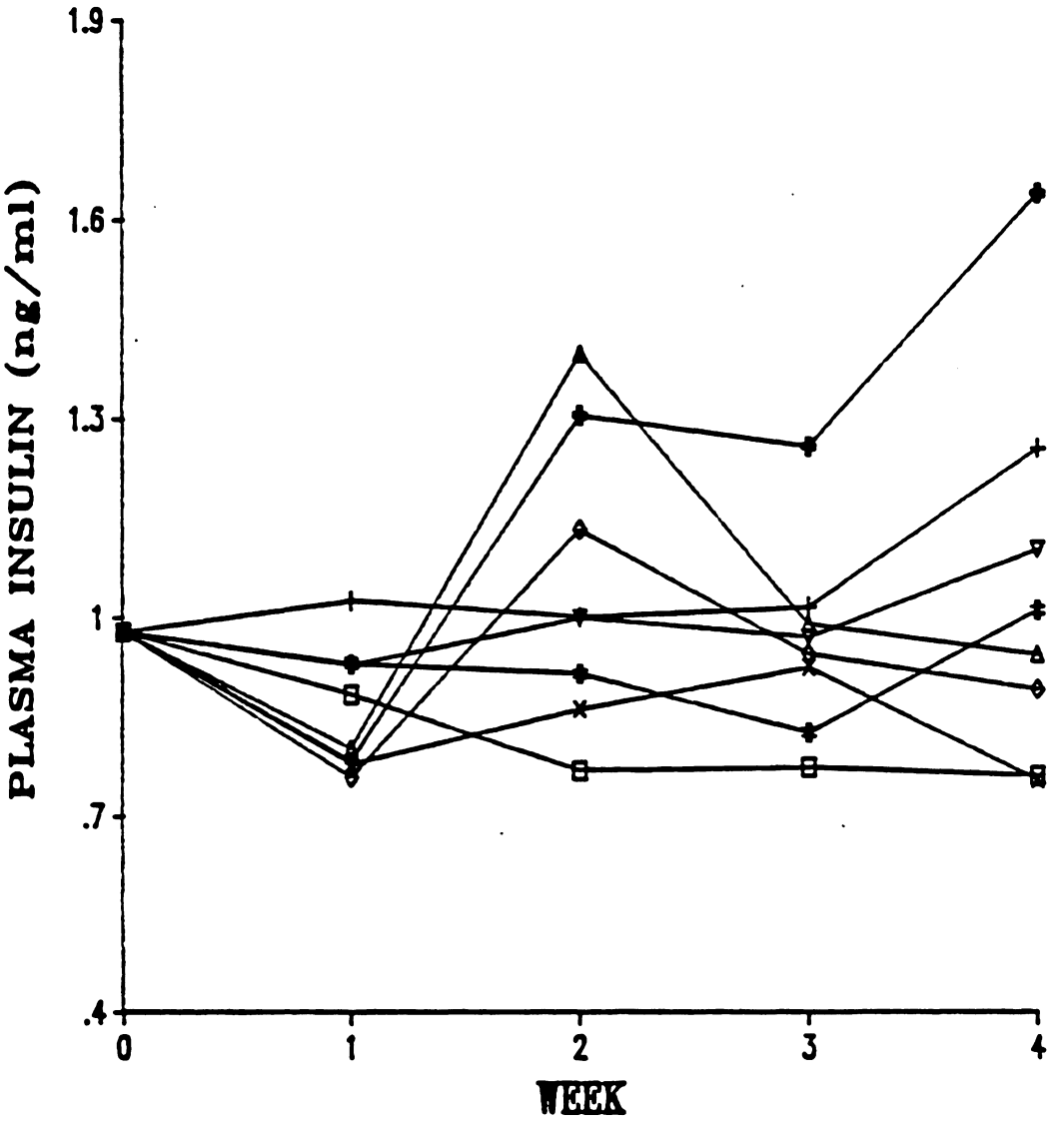

Figure 22. Plasma insulin concentrations of cows fed control (+), calcium stearate (×), tallow ($), and whole cottonseed (⊕) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added isoacids.

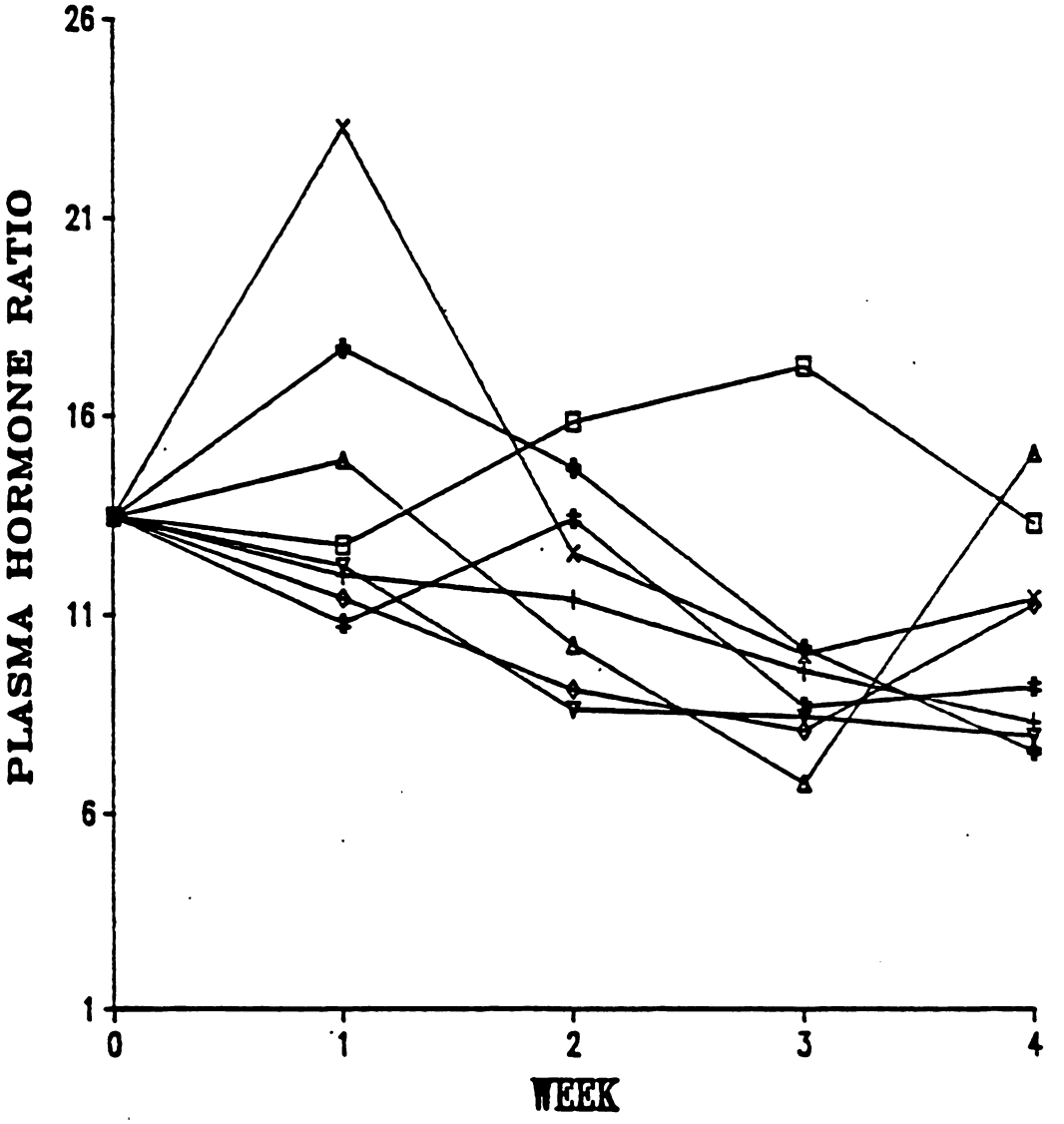

Figure 23. Plasma growth hormone to insulin ratio of cows fed control (+), calcium stearate (X), tallow ($), and whole cottonseed (✣) diets, and control (□), calcium stearate (△), tallow (◇), and whole cottonseed (▽) diets with added iscacids.

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