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THE USE OF CALCIUM TREATED ANIMAL FAT IN THE RATION OF DAIRY COWS

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THE USE OF CALCIUM TREATED ANIMAL FAT
IN THE RATION OF DAIRY COWS

by
Sadi Shalan Khalaf

A Thesis Submitted to the Faculty of the
DEPARTMENT OF ANIMAL SCIENCES
In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE
WITH A MAJOR IN DAIRY SCIENCE

In the Graduate College
THE UNIVERSITY OF ARIZONA

1985
STATEMENT BY AUTHOR

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ABSTRACT

An experiment utilizing 25 lactating dairy cows was designed to study the effects of calcium treated animal fat on milk production and milk composition. Five experimental rations were compared. All contained 50% alfalfa hay plus one of the following: 50% dairy concentrate (control), 44% dairy concentrate + 6% calcium treated animal fat (4% TF), 38% dairy concentrate + 12% calcium treated animal fat (8% TF), 46% dairy concentrate + 4% animal fat (4% F), 42% dairy concentrate + 8% animal fat (8% F).

There were no significant differences in milk production and milk composition among the experimental rations. Digestibilities of ADF, Cellulose, EE, and CM were significantly changed by feeding the calcium treated animal fat or animal fat. Serum total lipid and serum total cholesterol were significantly increased on 4% TF and 8% TF diets compared to the control diet. Changes in rumen VFA, fecal soaps, serum fatty acids, and milk fatty acids were also observed.
INTRODUCTION

High producing lactating cows are energy deficient animals at their peak of lactation. In early lactation dairy cows experience difficulty in consuming sufficient feed to meet energy needs for high milk production due to gut fill (9) and low appetite (23). To meet their extra energy demands, they tend to mobilize body reserves which are replenished later in lactation when milk output is lower and appetite higher. Storage and release of body reserves in this way is not as efficient as meeting the cow's needs directly from the diet (209) and may lead to metabolic upsets such as acetonemia and fertility problems if body weight loss is excessive. Increasing density of dietary energy with conventional carbohydrate diets may reduce milk and fat yields and energetic efficiency with no net increase in energy consumption (97, 167).

Fats and oils are useful dietary ingredients for increasing energy intake, milk yield and energetic efficiency. However, the use of fat supplements in rations for dairy cows has been limited to levels which do not induce metabolic changes in the rumen and lead to impaired digestion and reduced feed intake. The usual level of supplemental fat in ruminant diets is not more than 5%. Treating the dietary fats in a way to prevent its adverse effect on rumen metabolism may help to increase its utilization in dairy rations. More recently, Chalupa et al (38) found that calcium salts of tallow or vegetable fat reduced
the ruminal acetate/propionate ratio by 16%, whereas triglycerides of tallow reduced it by 48%. Therefore this technique may provide a new means of using large quantities of dietary fats and oils without adversely affecting rumen metabolism. The present investigation was carried out to determine the effect of calcium treated animal fat on milk production and milk composition of lactating dairy cows.
REVIEW OF LITERATURE

Influence of Dietary Fat on Feed Intake

Beitz and Davis (18) studied the effect of feeding cod liver oil and high-grain restricted roughage diets to high producing lactating cows. They indicated that the addition of 225 g/day of cod liver oil to a concentrate mixture did not alter grain consumption. Lack of feed intake depression with the free form of cod liver oil possibly was due to the low level of supplementation (193). Normal feed consumption was obtained by feeding milking cows different types and different levels of vegetable oils (15, 83, 104, 146, 156, 162, 176, 187).

Macleod and Wood (111) compared a standard dairy ration with a low-fat basal diet supplemented with or without hydrogenated tallow or soybean oil. Voluntary feed intake was not affected by the addition of tallow or soybean oil.

Depression of feed intake in association with an increasing level of added dietary lipid has been shown in the literature (24, 59, 72, 76, 96, 153, 164, 171, 177, 191, 223). Concentrate mixtures containing 50% formaldehyde-protected tallow fed to cross-bred beef heifers as 20% of their diet failed to be consumed at levels high enough to get gains of 0.5 kg/head/day (164). Yet, these gains were obtained when the protected supplement was reduced to 18%. Either lack of palatability or metabolic parameter changes associated with increased fat
absorption was then suggested as the cause of reduced feed intake at high levels of dietary fat feeding. Bines et al (24) found both roughage and concentrate consumptions were reduced by feeding lactating cows 1.7, 3.0 or 5.0 kg/day of a protected soybean-tallow supplement. They indicated that incomplete protection of the supplement was behind this reduction in feed intake. The change in rumen volatile fatty acid (VFA), reduction in fiber digestibility and reduction in the output of short chain fatty acids of milk fat led these workers to their conclusion.

Kowalczyk et al (99) suggested that the detrimental effects of dietary lipid is related to the change in rumen fermentation and not to the limited capacity for absorption by the animal. They showed that voluntary feed intake of sheep was reduced when dried grass and a high-fat supplement in a dry form was fed. But, when the high-fat supplement was fed in a liquid suspension to bypass the rumen, voluntary feed intake was not affected. Recently, Chalupa et al (38) indicated that the acetate/propionate ratio of rumen liquor was reduced 48% by feeding 10% tallow triglycerides, whereas feeding the same level of tallow triglycerides or vegetable fat in the form of calcium salts reduced the acetate/propionate ratio by only 16%. Calcium soaps of tallow, fat blend or soya oil however, had no effect on the pattern of feed intake when fed at 12% of the concentrate ration to high producing lactating cows (150).

Reduced availability of calcium and magnesium for rumen microorganisms due to the formation of fecal soap with high levels of dietary lipid was also suggested as a cause of reduced voluntary feed
intake (96). Feed intake was reduced by ensiling 12% partially hydrolyzed animal and vegetable fat (HEF) with corn plant materials and fed to steers (96). The reduction in feed intake was alleviated by addition of 1% limestone. Moreover, Ørskov et al (144) indicated that dried grass fed to early-weaned lambs with either 6.5 or 13.0% tallow caused a linear reduction in intake and fiber digestibility. The reduction in feed intake was partially alleviated by addition of 1.5% of urea. Consequently, it was hypothesized that the depression in feed intake by dietary fat was possibly related to a reduced concentration of NH₃ caused by the inhibition of certain types of bacteria and protozoa and possible coating with free fatty acids. Sutton and Johnson (202) on the other hand reported that feed refusal was associated with high levels of cereal grains and lower rumen pH and higher rumen levels of lactic acid. Changes in rumen pH with the feeding of low (111), intermediate (126) or high levels (59) of fat were not obtained however. Macleod et al (113) showed that a concentrate mixture supplemented with either 21 or 32% protected animal tallow and fed ad libitum to dairy cows resulted in low dry matter intake from concentrate because of its low acceptability. The reduction in grain consumption was compensated by increasing roughage intake. This response was not observed elsewhere in the literature.

In disagreement with similar studies (24, 177) Wrenn et al (224) reported no reduction in feed intake by feeding milking cows 25% of their grain diet as protected-tallow. Instead, cows on the protected supplement tended to eat more than the cows on the control diet.
Influence of Dietary Fat on Nutrient Digestibility

Fiber Digestibility

Free and possibly protected forms of fats and oils have been known to depress crude fiber digestion by ruminants (24, 33, 34, 54, 56, 65, 67, 90, 92, 93, 96, 100, 108, 110, 120, 134, 144, 159, 215, 220). The exact mechanism whereby dietary fat reduces crude fiber digestion is not known. However, many theories have been developed. Some of these theories were reviewed by Figroid (72) and Devendra and Lewis (56). One theory is the coating effect of the lipid on the fiber portion of the diet protects it from microbial attack in the rumen. This theory was proposed by Brooks et al (34) and supported by the works of Erwin et al (67) and Ward et al (215). Brooks and his coworkers (34) found that cellulose digestion in-vitro was reduced 40-94% by the addition of 10-170 mg of corn oil to 1g of dry matter containing 50% cellulose. Reduction in cellulose and protein digestibility was also obtained by the inclusion of 32-64 g of corn oil to a sheep basal diet of cottonseed hulls and casein (34). In addition, Brooks et al (34) showed that 32-64 g of lard also lowered cellulose digestion and that the depressing effect of dietary lard and oil was partially alleviated by the addition of alfalfa ash. It was hypothesized then (34) that alfalfa ash improved cellulose digestion probably by either its buffering capacity or by helping to emulsify dietary fat and prevent it from coating the fiber part of the diet. Later, Ward et al (215) studied this theory and found that 2.4% (of the ration) corn oil did not depress fiber digestibility when it was only added to the concentrate diet, but it
did depress fiber digestion when added to the entire ration which contained cottonseed hulls. Nevertheless, this appeared to contradict the results of Brethour et al (33) who found that 15% animal fat significantly reduced digestibility of dry matter and organic matter, and weight gains of sheep. Similarly, they found that 10% corn oil also reduced weight gains of sheep regardless of whether the corn oil was absorbed by the concentrate or the roughage portion of the diet.

The second theory suggests that lipid supplementation causes a modification or reduction in the activity of rumen microorganisms concerned with cellulose digestion. This mechanism was hypothesized by White et al (220) who found that cellulose digestibility was reduced by inclusion of 5% corn oil in the diet of sheep. Complete recovery of cellulose digestion was not accomplished until 17 days after withdrawal of the oil from the diet. More recently, the coating effect and the inhibition of a specific type of rumen bacteria by dietary lipid was investigated by McAllan et al (120). They proposed that if the effect of dietary lipid in the rumen was on specific bacterial species, that different types of structural carbohydrate would not be affected to the same extent. However, if the effect of dietary lipid was to coat the fibrous portion of the diet, it would be suggested that all structural sugars would be affected similarly. To investigate the previous two hypotheses, they fed sheep fitted with rumen re-entrant duodenal cannulas 200 g of hay and 400 g of concentrate mixture alone or supplemented with either 40 g of linseed or coconut oil. The oils were given in two forms; free or protected with formaldehyde-casein. They found that the sugars entering the duodenum contributed by the
microorganisms were: all the rhamnose and ribose and 51%, 24% and 35% of the mannose, galactose and starch-glucose respectively. Sugars of diet origin were: all the arabinose, xylose and cellulose-glucose. Digestion coefficients of sugars between the mouth and duodenum were: 95%, 66%, 67%, 62%, 45% and 51% for starch-glucose, mannose, arabinose, galactose, xylose and cellulose-glucose respectively for the basal diet with no oil. The corresponding values for the diet with free oil were: 95%, 55%, 38%, 55%, 1% and -2% respectively. No significant difference between the effect of linseed oil or coconut oil were observed. Protected lipids also tended to depress structural carbohydrate but to a lesser extent compared to free oils. Consequently, it can be noted that galactose and mannose, although structural sugars, were not affected significantly by the addition of free oils. These authors (120) suggested that the smaller effect of free oils on these sugars was probably due to their presence as side chains, large hemicellulose units or as more soluble smaller galactomannan chains. The digestion of some arabinose and little or no digestion of the cellulose-glucose or hemicellulose-xylose in the presence of free oils led these authors to suggest that there was a complete inhibition in the digestion of certain types of carbohydrates. Also the hemicellulytic and cellulytic bacteria were either eliminated or their glycan hydrolase activity was completely inhibited. Although no clear-cut differences were found concerning the reduction in structural carbohydrate digestibility with free oils, they suggested that the results favor the change in microbial activities over the coating effect mechanism. Furthermore, they indicated that the protected oils had intermediate effects between the free oil diets and the
diets with no oils. They attributed the protected oils effect to either incomplete protection or that fiber digestion is affected even by the protected forms.

The modification in rumen microorganisms theory could also be supported by faunation and defaunation experiments (55, 103). Czerkawski (45, 48) in a series of experiments with sheep found that increasing linseed oil or linseed fatty acid feeding resulted in a marked reduction in the total number of protozoa in the rumen and this was associated with an inhibition of methane production, a reduction in the molar percentage of rumen acetate and butyrate and an increase in the molar percentage of rumen propionate. Ikwuegbu and Sutton (90) also found that basal sheep diets supplemented with 13, 26 or 40 ml of linseed oil caused a reduction in fiber digestibility, almost completely eliminated protozoa from the rumen, especially at the higher level, and reduced the ratio of acetate to propionate in the rumen fluid.

The relationship between the bacteria and the ciliate protozoa in the rumen of sheep fed a purified diet was revealed by Kurihara et al (103). They used two sheep with or without ciliate protozoa and fed them a diet composed of wood-pulp cellulose, corn starch and soybean protein. They found that the faunated sheep (with protozoa) had half the number of total bacteria, but cellulytic bacteria were higher and amylolytic bacteria were lower compared to unfaunated (without protozoa) sheep. They also showed that cellulose digestion in the faunated animal was two times higher than in the defaunated animal. They attributed the increase in cellulose digestion in the faunated animal
not only to the increase in the number of cellulytic bacteria but also
to the fact that the bacteria in the faunated sheep were entirely R.
albus which are known to have a greater cellulytic activity. Furthermore, faunated sheep had more acetic acid and butyric acid and less
propionic acid in the rumen liquor when compared to defaunated sheep.
They indicated that the faunated rumen contained greater numbers of
Ruminococci bacteria which produce mainly acetic acid, whereas
Bacteroids, which constitute the major part of the flora of the un-
faunated rumen produce acetic acid as well as succinic acid which can
be rapidly converted to propionic acid. The results of Kurihara et al
(103) were supported by the work of Demeyer and Van Nevel (55) in-vitro.

The third theory involves some of the macro elements availa-
bility during lipid feeding. It suggests that during dietary lipid
supplementation there is a reduction in fiber digestibility coupled with
an excessive excretion of fecal soaps which may reduce the availability
of calcium and magnesium for normal activity of rumen microorganisms.
Ward et al (215) indicated that the detrimental effect of corn oil on
ration digestibility was completely reversed by addition of alfalfa ash
if the level of corn oil did not exceed 5% of the total ration. There
was a partial effect when the level of corn oil was higher. Likewise,
White et al (220) reported that the depressed digestibility of cellulose
which resulted from the addition of corn oil was reversed by the ad-
dition of 30 g of alfalfa ash, 4.4 g of calcium or 4.4 g of calcium plus
0.86 g of phosphorus per kg. The addition of 0.86 g of phosphorus
alone or a trace mineral mixture of Cu, Mo, Mn, Co, Fe, Zn, and B did
not restore the depressed digestibility of cellulose. The mineral
theory was also supported by the works of Davison and Woods (52, 53), Johnson and McClure (96) and Devendra and Lewis (56). Johnson and McClure (96) found that silage containing 8% corn oil had significantly reduced digestibilities of organic matter (OM), dry matter (DM) and cellulose in comparison to 4% corn oil silage or 8% corn oil silage plus 1% limestone. Finally, 8% beef tallow or maize oil fed with 2.3 or 4.8 g/kg of calcium resulted in low digestibility of fiber (56). But, when the calcium was increased to 22.9 g, fiber digestibility improved for the diet containing maize oil.

The fourth theory indicated that lipid supplementation may result in an inhibition of the activity of the microorganisms of the rumen. Nieman (138) concluded that fatty acids could be adsorbed by the cell wall and alter its permeability leading to inhibition of the growth of the microorganisms. He also added that the inhibition of growth was caused by both saturated and unsaturated acids, the effects of unsaturated fatty acids increased with the number of double bonds and the effects of saturated fatty acids were maximal when there were twelve or fourteen carbon atoms in the molecule. Maxcy and Dill (118) indicated that fatty acids are weakly adsorbed on bacteria and this adsorption was associated with the inhibitory process. Camein and Dunn [cited by Devendra and Lewis (56)] suggested that the inhibitory effect of saturated fatty acids of 12 to 20 carbons on the growth of lactic acid bacteria was as an antimetabolite. Czerkawski et al (47) on the other hand, indicated that, while linseed oil inhibited the growth of gram-positive methanogenic bacteria, the cellulytic bacteria were allowed to grow normally.
Surface active agents could be another factor influencing the utilization of lipids and fiber (56). Fedde et al (70) reported that the absorbability of 20% beef tallow fed to chicks was increased significantly by the addition of 0.5% or more of ox bile. They suggested that exogenous ox bile may directly assist in fat absorption and/or it may stimulate the liver cells to secrete more bile acids which would aid in fat absorption. Bolton (30) also found that non-ionic detergent improved fat digestion in chicks. Conversely, Moody (126) with lactating cows found that the addition of 5.5% tallow (to the concentrate portion of the ration) plus 0.5% sucroglyceride (non-ionic surface active agent) did not enhance the digestibility of lipid over a diet containing 6% tallow. Both diets however reduced digestibility of feed DM, protein, and ADF. Moreover, two types of non-ionic detergents added as 0.1% of the diet of sheep containing corn oil or HEF fat failed to significantly increase the digestibility of total lipids and crude fiber (56). The failure of these detergents was attributed to some possible factors such as, breakdown in the rumen, alteration of microbial activity, inhibition of pancreatic lipase, the use of inappropriate surface active agents and finally incorrect levels of lipid, detergent or fiber in the diet.

Finally, Devendra and Lewis (56) provided a possible model by which dietary lipid has a physical wetting effect on the fiber part of the diet which leads to the depression in its digestibility. They indicated that with the diet containing no lipid supplement, the fiber surface in an aqueous medium is hydrophilic to the microorganisms and this could lead to higher fiber digestion. By the addition of lipid
to the diet, a complete physical wetting of the fiber will result and the surface become hydrophobic to the microorganisms which reduces the angle of contact between the surface of the fiber and the microorganisms and causes the depression in fiber digestibility.

Dry Matter Digestibility

Free forms of dietary fats and oils, particularly at high levels of supplementation are known to reduce DM digestibility (33, 65, 67, 90). Erwin et al (67) indicated that the decrease in the digestibility of some ration nutrients with the addition of fat is due to the coating effect of fat on the fiber component of the diet, thus preventing the cellulytic microorganisms from freely degrading the fiber portion of the diet. Failure to improve rate of gain of steers at high levels of fat feeding was concluded to be due to the reduction in dry matter and crude fiber digestibilities associated with fat feeding (65). Ikwuegbu and Sutten (90) found that basal sheep diets containing 200 g of hay and 400 g of concentrate had reduced organic matter digestibilities when supplemented with 13, 26 or 40 ml/day of linseed oil.

The depressing effect of neutral fats on ration digestibility appears to be due to their fatty acid content (54, 182). Davison and Woods (52) in studies conducted to investigate the effects of C2-C18 fatty acids and glycerol upon cellulose digestion, reported that the addition to lamb rations of 5% corn oil, a 5% mixture of fatty acids, 1% lauric acid, 5% stearic acid, or 5% oleic acid significantly depressed digestibility of DM and organic matter (OM). Steele and Moore (182) on the other hand found that myristic acid had no effect on
total DM digestibility whereas palmitic acid and stearic acid depressed DM digestibility.

Lipid feeding in association with the mineral content of the diet has also been studied (33, 52, 53, 96, 215). Ward et al (215) showed that the detrimental effect of corn oil on ruminant ration digestibility was completely reversed by the addition of alfalfa ash if the level of corn oil did not exceed 5% of the total ration and only partially alleviated the effect if the level of corn oil was higher. Brethour et al (33) showed that alfalfa ash did not prevent the depressing effect of 15% animal fat on ration OM and DM digestibilities. Addition of either calcium carbonate or calcium chloride did remove the depressing effect of corn oil on ration DM digestibility (52, 53). However, rations containing alfalfa hay appeared to be unaffected by the addition of corn oil or calcium (52). In contrast to calcium addition, saponification of corn oil with KOH (52) or addition of magnesium carbonate (53), sodium or potassium bicarbonate (33) did not alleviate the depressing effect of corn oil (52, 53) or animal fat (33) on ration digestibility. Johnson and McClure (96) reported that the addition of 1% limestone removed the detrimental effect of 8% corn oil on DM and OM digestibilities of silage.

Low levels of fat fed to steers (69) or lactating cows (126) were shown to have no effects on DM digestibility of the diet, yet other studies (59, 145, 146) involving high levels of dietary fats were also reported to have no effect on DM digestibility.

Several studies involving the use of formaldehyde-protected fat and its effect on ration digestibility are also reported elsewhere (24,
Sutton et al (203) reported that OM digestibility of the diet of sheep was depressed by the addition of 40 ml/day of free linseed oil. However, when the linseed oil was protected against ruminal hydrolysis the OM digestibility was improved but the depressing effect was not completely eliminated. Reduction of DM digestibility with the feeding of a protected supplement was also reported by others (24, 117, 134). Murphy and Morgan (134) noticed that both protected and unprotected tallow increased digestible energy and metabolizable energy when added to the diet of steers. Both, however, tended to reduce DM digestibility of the diet. Although Kronfeld and Donghue (100) indicated that rations containing 18% protected tallow had increased DM digestibility when fed to Guernsy heifers, others (59, 171) found no effect of protected fats on DM digestibility of steer or lactating cow rations.

Gross Energy Digestibility

Esplin et al (69) reported slight improvement in the digestibility of gross energy by the addition of 4% tallow or hydrolyzed vegetable and animal fat to a typical fattening ration of 30% alfalfa hay, 57% grain, 7% molasses, 5% cottonseed meal and 1% dicalcium phosphate. Smith et al (176) indicated that energy digestibility increased with an increasing dietary level of whole cottonseed in the diet of lactating cows. The levels of whole cottonseed used were 0, 5, 10 and 25% of the complete ration. Conversely, a reduction in energy digestibility with the addition of fat was observed by others (24, 31, 44, 90, 134). Cuitun et al (44) for instance found that 6% free safflower oil fed to steers along with 80% concentrate and 5% alfalfa hay
reduced energy digestibility compared to a diet containing 6% formaldehyde-treated safflower oil. Reduction in the digestibility of energy with increasing dietary protected fat was also noticed by Bines et al (24) and Murphy and Morgan (134). Mature sheep fed basal diets of 200 g of hay and 400 g of concentrate showed low levels of energy digestibility when their diet was supplemented with 13, 26 or 40 ml/day of linseed oil (90). Other studies involving the feeding of protected fat (59, 171) or free fat (145, 146) indicated no significant change in the digestibility of energy by the addition of fat into the diet.

Crude Protein Digestibility

Reduction in protein digestion resulted from inclusion of 32-64 g of corn oil into a sheep basal diet of cottonseed hulls and casein (34). Bradley et al (31) studied the interrelationship between dietary fat and urea in steer rations. Addition of 5% fat to a control concentrate ration reduced digestibility of DM, energy and nitrogen-free extract (NFE). Digestibility of cellulose was not affected because of the low level of the added fat. The inclusion of urea along with animal fat also depressed digestibility of crude protein. Phillips and Roberts (159) indicated that oral oil compared to duodenal oil administration tended to increase nitrogen retention possibly by reducing NH₃ losses. They also added that rumen fermentation was improved by oral protein-oral oil administration. Depression in crude protein (CP) digestibility was also observed with diets supplemented with a formaldehyde-treated protein-fat supplement (24, 59, 117). The decrease in CP digestibility with this type of supplement was attributed to lower degradability of the supplement inside the rumen (117). On the other
hand, Macleod et al (113) reported no change in CP digestibility when feeding lactating cows concentrate mixtures containing up to 32% protected tallow. Untreated tallow or blended animal and vegetable fat fed as either 6% (126) or 10% of the concentrate mixture (146) had no effect on CP digestibility. This was in disagreement with the result of Perry and Stewart (157) who found a reduction in CP digestibility with the addition of 3% animal fat. Improvements, however, in the digestion of protein was noticed by others (100, 145, 176). Palmquist and Conrad (145) reported increased digestibility of nitrogen when fat and protein were increased in the diet of lactating cows. Improvement in the digestibility of protein as a result of feeding 18% protected tallow was noticed by Kronfeld and Donghue (100). Others (24, 59, 117) found no improvement.

Mineral Digestibility

In general, dietary lipid in both free and protected forms tended to reduce mineral digestibility (24, 54, 94, 100, 157, 171). Kronfeld and Donghue (100) indicated that the apparent reduction in Ca and Mg digestibilities was due to increased fecal soap formation. The depressed apparent digestibility of Ca, Mg and P with increasing dietary protected fat feeding appeared to be negated after correction for endogenous losses (24). Moreover, Smith et al (176) found that the feeding of up to 25% whole cottonseed in the ration of dairy cows had no effect on Ca, Mg and P digestibilities.
Lipid Digestibility

The majority of the literature indicated higher lipid digestion in the diets when supplemented with fats and oils (54, 67, 69, 108, 113, 117, 126, 145, 159, 171, 176). However, it was noted that in all of these studies, lipids were determined using the ether extract procedure which cannot extract fecal lipids that are in the form of fecal soaps. It has been suggested by many studies (43, 72, 166, 214) that fecal soaps be considered in calculating lipid digestibility.

Figroid (72) used a chlorform-methanol-hydrochloric acid (CMH) extractions to determine lipid digestion in steers fed 0, 5, 10 or 15% animal fat with different levels of concentrate. He found that lipid digestibility was reduced with increasing dietary fat level. He also indicated that with increasing dietary fat feeding there was an increase in fecal soap formation. Sharma et al (171) on the other hand found that the apparent digestibility of ether extract and acid-solvent extract was higher on diets supplemented with formaldehyde-treated tallow. However, true digestibility of ether extract or acid-solvent extract was similar. Furthermore, Jenkins and Palmquist (94) reported normal fatty acid digestion by lactating cows fed alfalfa pellets, concentrate and silage, and supplemented with tallow fatty acids as calcium soaps.

Rumen Soap Formation

Brooks et al (34) noticed that the depressing effects of corn oil and lard on the digestibility of cellulose in the diet of sheep were partially alleviated by the addition of alfalfa ash. Davison and Woods (52), using sheep, indicated that the addition of CaCO₃ to either corn oil or saponified corn oil resulted in removal of the depressing
effects of corn oil on ration digestibility. Thereafter, Davison and Woods (53) reported that fatty acids from hydrolyzation of neutral fat in the rumen are available for the formation of soaps in the presence of calcium. These calcium soaps are insoluble and precipitate, and therefore are ineffective in depressing rumen microbial activity and thus allow the digesta to be normally fermented. The addition of magnesium carbonate (53) or saponification of corn oil with KOH (52) was ineffective in improving the digestibility of the diets that were depressed by the addition of fats. Similarly, Brethour et al (33) found that sodium and potassium form soaps which are soluble in an aqueous medium and also decreased cellulose digestion.

Recently, Jenkins and Palmquist (92) revealed some of the factors affecting the formation of insoluble soaps. In in-vitro experiments involving the inclusion of no fat, 10% tallow, 10% tallow plus 2% dicalcium phosphate, or 10% tallow plus 1.3% calcium chloride to a basal diet, they found that insoluble soap was increased by the addition of tallow. The addition of calcium also increased the formation of insoluble soaps, however, calcium from calcium chloride reacted faster compared to calcium from calcium phosphate, suggesting that the solubility of the cations is a factor in the formation of the soaps. They also added that the type of dietary fat and its component fatty acids was another factor affecting the formation of soaps. Increasing degree of saturation and chain length tended to increase the degree of insoluble soap formation. They indicated that the relationship between degree of unsaturated and formation of insoluble soaps may partially explain why unsaturated fatty acids have been known to be
more effective in inhibiting microbial activity than saturated fatty acids.

Davison and Woods (52) reported an increase in digestible energy by the addition of calcium to a diet containing corn oil. They suggested that fatty acids and calcium must dissociate to permit the absorption of fatty acids. Solubility of calcium was known to increase as it passes from the rumen to the abomasum where the pH drops to about 3 (77). Davison and Woods (53) suggested that as calcium and fatty acids pass into the small intestine in the dissociated state, calcium is probably absorbed before the pH returns to neutral. Fatty acids are then absorbed in the presence of bile salts or perhaps immediately upon passing from the abomasum. Some soaps of course escape in the feces, and there is some association of calcium with metabolic fatty acids to form soaps in the gut (28).

**Fecal Soap Excretion**

Crockett and Deuel (43) have suggested that the type of technique used to extract the lipid from the feces be considered when calculating fat digestibility. They found with rats that large amounts of undigested fat from hydrogenated lards was lost in the feces in the form of soap. In an experiment with lactating cows Ward and Reid (214) indicated that ether extract (EE) is a misleading procedure to estimate crude fat in the feces. Using the same grain mixture with varying mineral intake, they found no differences in total lipid content when the fecal fats were extracted by acid hydrolysis and chloroform. However, cows receiving high levels of calcium excreted a small proportion of fat in the feces as measured by the EE procedure. The difference
between the two results was attributed to the formation of calcium soaps from the high calcium in the diet which could not be extracted by the EE technique. The results of Ward and Reid (214) were supported by Roberts and McKirdy (166) who found that fecal EE and fecal crude fat percentages from feeding cattle rapeseed oil, sunflower seed oil, and animal tallow were: 2.77, 8.17; 3.56, 12.37; and 2.93, 8.10 respectively. Dijkstra (57) also reported that EE digestibilities of sheep fed 0, 5, 10, 15, or 20% fat were 52.9, 81.7, 86.8, 89.1 and 90.5% respectively, whereas digestibilities of lipid extracted by tetrachloromethane for the same rations were; 48.4, 64.9, 57.5, 61.4 and 69.6%. It was concluded (57) that the differences between the two digestibilities were related to the formation of calcium and magnesium insoluble soaps.

Increasing fecal soaps with increasing dietary fat feeding has been observed by others (69, 72, 96). Esplin et al (69) indicated that fecal soap did not represent a primary loss of dietary fat energy. However, the diets they fed to steers contained a relatively low level of fat. Conversely, Shell et al (175) fed diets to sheep that contained no fat, 8% untreated cottonseed oil or 8 and 12% formaldehyde-treated cottonseed oil and found that fecal soaps were higher on the diet with no fat compared to the other two diets. They suggested that increased lipid intake from the highly digestible fat of cottonseed oil led to their observations. On the other hand, Sharma et al (171) reported no difference in fecal soap excretion as a result of feeding 0, 10 or 15% of formaldehyde-protected tallow to milking cows.
Influence of Dietary Fat on Rumen Volatile Fatty Acids (VFA)

The relationship between molar percentages of rumen VFA and milk fat has been well documented (10, 12, 18, 37, 50, 98, 102, 151, 174, 191, 193, 211). Shaw et al (174) found a positive correlation (+0.64) between milk fat and rumen acetate and a negative correlation (-0.63) between milk fat and rumen propionate. Acetic acid and Beta-hydroxybutyric acid (BHBA) are known to be precursors for milk fat synthesis in the mammary gland (6, 107, 151, 155, 211). Linzell et al (107) using perfused mammary glands of goats showed that acetate and BHBA were extensively utilized for the synthesis of milk fatty acids of up to chain length C14 and to a smaller extent for the synthesis of C16:0. This was supported by the finding of Annison et al (6). In addition Palmquist et al (151) reported that about 50% of the fatty acids of milk fat are synthesized in the mammary gland of which 17% is from BHBA and the majority from acetate.

Rumen total VFA and molar percentages of rumen VFA are greatly influenced by the level and the physical form of the roughage and the concentrate of the diet. High-grain restricted roughage diets are known to reduce rumen acetate and increase rumen propionate (17, 18, 35, 181, 202). This was probably related to lower rumen pH associated with feeding of high-grain diets (12, 202). Balch et al (12) found that high-grain, low-roughage diets resulted in low rumen pH, increased total production of rumen VFA, and reduced percentage of rumen acetic acid. However, rumen propionate was not affected. Davis et al (50) investigated this phenomena in lactating cows and indicated that high-grain restricted-roughage diets caused low milk fat. The addition of
1.5 or 3% of the concentrate as bicarbonate (equal parts by weight of sodium and potassium) fed with 5 pounds of alfalfa hay produced a high acetate/propionate ratio and normal milk fat. They postulated that high-grain, low-roughage diets reduced saliva output which resulted in lowered buffering capacity of the rumen and lead to lower rumen pH. Low rumen pH favors the microorganisms that produce propionic acid which inhibits milk fat synthesis. Increasing the buffering capacity of the rumen by the addition of carbonate compensated for the reduction in milk fat. Nevertheless, Van Soest and Allen (211) partially attributed the reduction in milk fat which was associated with high-grain restricted-roughage diets to the antiketogenic effect of propionic acid. They found that low-roughage diets with high levels of concentrate increased rumen propionate and decreased blood ketone bodies. Rumen acetate was not significantly reduced as was butyrate. BHBA is a known precursor of milk fat. Propionic acid is first converted into succinate then to oxaloacetate which then condenses with acetyl coA. An increasing propionate/acetate ratio would divert acetyl coA from acetoacetate towards oxidation in the citric acid cycle and result in low ketone bodies and consequently low milk fat. In addition, it was indicated that acetate and propionate may compete over coA since both are bound to this enzyme. To support their conclusion, they indicated that feeding sodium acetate increased milk fat that was depressed, whereas feeding sodium propionate further reduced milk fat (10).

The effects of processing of the feed and the physical form of the diet on rumen VFA have been observed in the literature (17, 98, 173). Steer calves had low levels of rumen acetate and high levels of rumen
propionate when the hay in the diet was ground and pelleted and the corn was steam flaked (173). King and Hemken (98) found the same response in lactating cows. Replacement of half of the hay in the diet with linseed cake, decorticated ground nut cake, or flaked maize, or addition of maize starch or sucrose invariably reduced the molar percentage of rumen acetate (17).

Dietary fat influence on rumen volatile fatty acids is largely dependent on the level of fat, type of fat, physical form of the fat, and level of roughage in the diet of the animals.

Since fatty acids have been shown to effect rumen VFA as well as neutral fat (141, 169, 172, 183, 185), the influence was attributed to the fatty acids which are the product of fat hydrolysis in the rumen (79) and not the neutral fat itself.

Reduction in rumen acetate and an increase in ruminal propionate has been noticed by the addition of cod liver oil to the diet of cows (17, 37, 172, 193), steers (137) and sheep (204). This was suggested to be related to the polyunsaturated fatty acids of the cod liver oil (37). Brumby et al (37) showed that 300 g of native cod liver oil resulted in high propionate and low acetate in the rumen fluid of lactating cows. Yet, when the cod liver oil was hydrogenated, ruminal acetate significantly increased and ruminal propionate significantly decreased. Similarly, Storry et al (191, 193) found that formaldehyde-treated cod liver oil (193) or formaldehyde-treated coconut oil (191) failed to reduce the acetate/propionate ratio in the rumen fluid as did free cod liver oil or free coconut oil.
Sodium bicarbonate fed as 5.7% of steer diets composed of only concentrate failed to correct the depressed acetate/propionate ratio caused by the addition of 60 ml of cod liver oil (137). By comparison, 225 g/day of cod liver oil added to a normal diet of roughage and concentrate had no effect on acetate and propionate levels of the rumen fluid (18).

Depressed ruminal acetate/propionate ratios as a result of feeding highly polyunsaturated fatty acids was also reported by Palmquist and Conrad (145). They fed a grain mixture containing 35% ground raw soybeans and compared it to either a concentrate with no fat or concentrate with blended hydrolyzed fat. Mattos and Palmquist (117) found that 3.6 kg/day of untreated soyflour resulted in a significant reduction in ruminal acetate and a non-significant increase in ruminal propionate compared to 3.6 kg/day of formaldehyde-treated soyflour. Conversely, Perry and Macleod (156) and Larson and Schultz (104) reported no effect of raw soybeans and soybean oil or ground soybean on acetate and propionate concentrations of the rumen liquor.

The method of incorporating soybean oil in the concentrate mixture of lactating cows was investigated by Steele et al (187). They indicated that in a high roughage diet replacement of part of the starch in the concentrate with either 8% soybean oil or coarsely ground soybeans caused an increase in the molar percentage of rumen propionate and a reduction in the molar percentage of rumen butyrate. Only soybean oil reduced the molar percentage of rumen acetate. Likewise, Anderson et al (4) showed that 20% whole cottonseed had no effect on the acetate/propionate ratio of rumen fluid. By comparison, Ikwuegbu
and Sutton (90) indicated that rumen acetate and butyrate were reduced by 18% and 61% respectively by feeding sheep 13, 26 or 40 ml per day of linseed oil. In addition, at the 40 ml level, rumen propionate increased twofold.

The effect of individual fatty acids on rumen VFA has also been studied, particularly by the British workers. Shaw and Ensor (172) indicated that oleic acid (C18:1) and linoleic acid (C18:2) reduced rumen acetate and increased rumen propionate. The reduction in acetate and the increase in propionate by the addition of C18:1 has also been shown by Steele and Moore (185) and Selner and Schultz (169). On the other hand, a mixture of 10% saturated fatty acids (64% palmitic acid and 31% stearic acid) showed no effect on rumen acetate but did increase rumen propionate (185). This was also confirmed by Noble et al (141). Steele and Moore (183) found that 10% myristic (C14:0), palmitic (C16:0) or stearic (C18:0) acid had no effect on molar percentages of rumen VFA. But, 5% lauric acid (C12:0) tended to reduce the acetate/propionate ratio.

More recently, Chalupa et al (38) observed that the rumen acetate/propionate ratio was reduced 48% by addition of 10% tallow triglycerides, whereas the calcium salts of tallow or vegetable fat reduced the rumen acetate/propionate ratio by 16%. They suggested that long-chain fatty acid feeding in the form of calcium salts is an effective method to prevent the adverse effects of fat on rumen microorganisms. These authors also indicated that the slight effect of calcium salts of tallow and vegetable fat on rumen environment indicated that the salts were not completely inert in the rumen at high
levels of supplementation. The possibility of dissociation of some of the calcium salts of tallow or vegetable oil in the rumen could not be excluded.

Brown et al (35) indicated that 6% tallow or cottonseed oil showed no effect on molar percentages of rumen VFA. Both types of dietary fat indeed, were fed with high-or low-roughage diets to lactating cows. Their results were supported by other workers with both animal and vegetable fats (69, 95, 126, 160, 181, 197). Similar results were also noticed with formaldehyde-protected animal fats and vegetable oils (8, 117, 191, 192, 193). Conversely, Brumby et al (36) showed reductions in rumen acetate and increases in rumen propionate with increasing dietary feeding of a protected mixture of soybean flour and tallow to lactating dairy cows. Dinins et al (59) however, found that rumen acetate decreased and rumen propionate increased with protected supplements compared to the same level of unprotected supplements of safflower oil. Bines et al (24) showed that a protected soybean-tallow supplement fed to milking cows at levels of 1.7, 3.0 or 5.0 kg/day resulted in high rumen propionate and low rumen butyrate but had no effect on rumen acetate. On the other hand, Sharma et al (171) reported an increase in rumen acetate and a decrease in rumen propionate by feeding lactating cows 15% protected tallow.

Results regarding higher rumen VFA are inconsistent. However, butyric acid did tend to be lower with the feeding of both free (90, 141, 187, 193) and protected (24, 36) forms of dietary fats. Noble et al (141) found an increase in iso-butyrate and a decrease in n-butyrate from the addition of both 5 and 50% palmitic acid or 10% stearic acid.
Ruminal valeric and iso-valeric appeared to have a relationship between their presence in the diet and the availability of dietary protein for degradation in the rumen (35, 59, 104, 117). Diets rich in soybean meal (104) or casein (59) produced high levels of rumen valerate and iso-valerate. However, protecting the dietary protein with formaldehyde to prevent it from ruminal degradation resulted in low levels of rumen iso-valerate (117).

Influence of Dietary Fat on the Lipid Components of the Blood

Serum Total Lipids

Dietary lipid feeding has been shown to increase plasma total lipid (1, 29, 60, 72, 113, 114, 116, 143, 145, 149, 153, 162, 201, 224). An early University of Arizona study (201) conducted with lactating cows indicated that 7% rendered beef tallow added to the grain mixture increased serum total lipids from 470 to 606 mg/100 ml. Elevation in serum total lipids has been shown with lactating dairy cows (1, 113, 145, 149, 162, 201, 224), beef steers (29, 60, 72, 114, 143), sheep (116, 143) and dairy heifers (153). Increased serum total lipids was shown with both free (1, 29, 72, 114, 116, 143, 145, 153, 162, 201, 224) and protected (113, 116, 149, 224) forms of lipid.

Marchello et al (114) indicated that increasing dietary animal fat resulted in increased plasma total lipids of steers. However, higher levels of concentrate in the diet tended to decrease this effect (114, 115). It was reported that steers on either 5 or 15% fat had a 36% increase in serum total lipids, whereas steers on 10% fat had a 73% increase (114). Similar results were obtained by Park et al
(153) who found that 10, 20 and 30% whole sunflower seed diets fed to Holstein heifers increased serum total lipids. No significant difference between the 20% and 30% levels was noted. It was concluded that the 20% level (10% EE) was probably an effective limit to any increase in serum total lipids. Figroid (72) indicated that steers on 15% animal fat absorbed less fat than steers on 5 or 10% animal fat. No change in steers serum total lipids was obtained by feeding either 6% animal fat or safflower oil (115).

Serum Total Cholesterol

As with serum total lipid, serum cholesterol (total, esterified and unesterified) of ruminants tended to increase with dietary feeding of both protected and unprotected lipid (1, 26, 29, 58, 59, 60, 76, 81, 83, 84, 101, 112, 113, 153, 162, 171, 177, 224, 225, 226, 227, 228). Storry et al (199) showed that decreasing dietary fat feeding to dairy cows from 430 to 170 g/day resulted in decreases in free and esterified cholesterol of blood plasma. Garrett et al (76) reported increases of 3 to 4 times in plasma cholesterol of beef steers when fed high levels of protected-vegetable oil or tallow. Greater response of serum cholesterol to protected fat than unprotected fat was reported (224). Macleod et al (112) found that soybean oil gave higher values for plasma cholesterol esters than hydrogenated tallow in lactating cows. Lower digestibility of hydrogenated tallow compared to soybean oil was suggested to be the cause of such response. Wrenn et al (226) reported that total plasma cholesterol increased with increasing dietary protected sunflower-soybean oil feeding with lactating cows. At higher dietary oil feeding plasma total cholesterol
was increased to facilitate the transport of increased plasma lipids. On the other hand, Dinius et al (58) reported an inverse relationship between the level of protected oil and serum total cholesterol. They found that diets containing 10% protected safflower oil had the highest level of serum total cholesterol, diets of 20% protected safflower oil were intermediate and diets of 30% protected safflower oil had the lowest level of serum total cholesterol. To explain their finding, these authors (58) indicated that high levels of protected oil caused a diarrheic effect and lowered feed intake leading to reduced absorption of lipid and lowered rate of passage of food which gave rumen microorganisms a greater opportunity to utilize the protected oil which then caused reduced absorption of lipids. Adams et al (1) showed that 10% of the concentrate mixture fed to lactating cows as animal tallow or vegetable oil significantly increased serum cholesterol. Feeding a mixture of soysterol and animal sterol on the other hand, tended to reduce serum cholesterol (1). When either one of the sterols was fed alone there was no effect on serum cholesterol. It was suggested that the synthesis of cholesterol which also occurs in the small intestine was reduced at the small intestine site by the mixture of plant and animal sterols.

Inhibition of cholesterol and triglycerides synthesis following the withdrawal of oil feeding has been shown by Smith et al (177). Dairy rations containing 15 or 30% protected tallow significantly increased the concentration of plasma triglycerides and cholesterol. However, these plasma components were depressed below that of the control diet 5 weeks after changing to the postexperimental diet of
no fat. Thus indicating that synthesis of these plasma parameters was inhibited by the feeding of protected tallow diets.

According to Nestel et al (136) hypercholesterolemia in fat-fed ruminants is largely due to increased intestinal synthesis of cholesterol and possibly due to decreased fecal excretion of bile acids. In a study conducted with sheep and goats, they indicated that synthesis of large amounts of cholesterol facilitates the absorption of long-chain fatty acids. The proximal part of the small intestine was found to be the major site of sterol synthesis in sheep. It was also found that dietary fat enhanced sterolgenesis in the small intestine both in-vitro and in-vivo (136). Conversely fat addition tended to depress sterolgenesis in the liver in-vitro (136). Nestel and his coworkers (136) also indicated that intestinal sterolgenesis was observed with several varieties of fats, however, palm oil feeding was shown to be the most stimulatory fat in this regard. In addition, they indicated that the increase in the synthesis of cholesterol in the small intestine which helps in the transport of the absorbed triglycerides in the chylomicrons led to the inhibition of the synthesis of cholesterol in the liver.

Moreover, Rafalowski and Park (162) found an inverse relationship between blood cholesterol and blood protein concentrations in lactating cows fed high levels of whole sunflower seeds. It was postulated that the reduction in blood protein is possibly related to some compositional changes in lipoproteins that are associated with the transport of increased levels of lipid in the plasma. This relationship however, was not confirmed later on by the study of Park et al
They explained (153) the controversy as resulting from the difference between lactating cows and heifers in functional and physiological status in relation to the composition and metabolism of apolipoproteins.

Plasma Triglycerides (TG), Phospholipids (PL) and Non-Esterfied Fatty Acids (NEFA)

Plasma TG and PL were reduced when the dietary fat in the diet of dairy cows was reduced from 430 to 170 g/day (199). Bitman et al (26) showed that blood TG and NEFA were increased threefold by feeding lactating cows protected polyunsaturated oils. Digestibility of dietary fats has been shown to effect the extent of the increase in blood lipid fractions (112). Macleod et al (112) reported higher plasma TG and PL when feeding lactating cows soybean oil compared to hydrogenated tallow. Increases in plasma fractions of TG, PL, and NEFA with the addition of dietary protected and unprotected fats and oils have been reported by others (24, 81, 83, 84, 113, 145, 153, 171, 177, 227). Goering et al (83) found that feeding protected soybean oil produced higher plasma NEFA than normal soybean oil. Although plasma PL of cows were significantly increased by feeding 250 or 500 ml/cow/day of linoleic acid (C18:1), plasma TG and NEFA were not changed (169). Non-significant changes in the plasma fractions of TG, NEFA and PL were also observed by feeding 454 ml of oil containing low (13%) or high (49%) levels of trans C18:1, however, PL concentration tended to increase with high trans acid feeding (169).
Plasma Fatty Acids

The relationship between plasma fatty acids and milk fatty acids is of particular interest since about 50% of milk fatty acids are derived from plasma fatty acids (151). Milk fatty acids of chain length of C4-C14 are synthesized in the mammary gland from acetate and BHBA (6, 91, 102, 107, 151). Part of the C16 and possibly all of the C18 fatty acids are taken up by the mammary gland from plasma sources (6, 16, 19, 21, 107, 151). Fatty acids of TG from chylomicrons and low-density lipoproteins of the plasma have been shown to be the sources of long-chain fatty acids for the mammary gland (6, 16). Annison et al (6) indicated that 60% of milk fat is derived from plasma triglycerides. Palmquist et al (151) reported that 40% of C16:0 and all C18 fatty acids are derived from sources other than mammary gland synthesis. No arteriovenous differences were found in the mammary gland of goats for phospholipids, sterols or sterol esters and their fatty acids (6). Plasma NEFA however, were found to be in equilibrium with the NEFA pool in the mammary gland. This pool of NEFA in the mammary gland is the produce of the hydrolysis of plasma triglycerides as a first step in triglyceride uptake by the mammary gland (6, 218, 219). Blood triglycerides are taken up by the mammary gland tissue and partially or completely hydrolyzed. The resulting fatty acids are then incorporated into new triglyceride molecules in the mammary gland (16, 155).

Based on this relationship between plasma and milk fatty acids, any changes in the plasma fatty acids, particularly the triglyceride fraction, could be reflected in the composition of the milk.
Under normal dietary conditions, the major fatty acids of the cow plasma triglycerides are palmitic (C16:0), stearic (C18:0) and oleic (C18:1) (62). Increases in plasma lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids with increasing dietary intake were observed (199). Despite the extensive hydrogenation that occurs in the rumen (5, 22, 79, 207), polyunsaturated plasma fatty acids were increased by increasing their dietary feeding or their intraruminal infusion (129, 142). This was possibly due to their having escaped hydrogenation (129). Moore et al (129) showed that replacement of a hay diet containing high concentrations of C18:2 and low concentration of linolenic acid (C18:3) with a hay diet containing high C18:3 and low C18:2 resulted in high concentrations of C18:3 and low concentrations of C18:2 in plasma cholesterol esters and phospholipids of sheep. Plasma triglycerides however, were higher in C18:0 and lower in C16:0. Plasma NEFA composition was not affected. Intraruminal infusion of 60 g/day of C18:3 resulted in increased levels of C18:3 in plasma cholesterol esters and phospholipids and increased levels of C18:0 in plasma triglycerides. Intraruminal infusion of 60 g/day of C18:2 increased C18:2 concentration in plasma cholesterol esters and phospholipids. Infusion of either C18:3 or C18:2 did not change plasma NEFA. The authors also indicated that increased concentrations of C18:0 in plasma triglycerides with intraruminal infusion of C18:3 but not with infusion of C18:2 may suggest that the rumen microflora hydrogenate C18:3 to C18:0 more readily than they hydrogenate C18:2 to C18:0.
The results of Moore et al (129) regarding the change in plasma triglyceride fatty acids composition with the administration of C18:2 or C18:3 were not supported by the works of either Moore et al (130) or Noble et al (142). Moore et al (130), also with sheep, found that intraabomasal infusion of emulsions of linseed oil, maize oil, or linoleic acid (C18:2) resulted in higher concentration of C18:3 and C18:2 in plasma triglycerides within 1.5 hours of the infusions. The increase in C18:3 and C18:2 in plasma triglycerides was associated with decreases in the concentration of C18:0 and C16:0 with no change noted in C18:1. The increase in the concentration of C18:3 and C18:2 in plasma cholesterol esters and phospholipids however, did not begin until 24-25 hours and 8-9 hours respectively following the infusion. They suggested that the absorbed C18:2 and C18:3 are transported as triglycerides to the liver and thus appear in high concentrations within a short time after infusion. When these triglycerides enter the liver they are partially or completely hydrolyzed with the polyunsaturated fatty acids being preferentially utilized for the synthesis of phospholipids and cholesterol esters but not for the resynthesis of triglycerides. These authors (130) provided no explanation for the conflicting results they obtained in both studies (129, 130) regarding triglyceride fatty acids. They did suggest that ruminal hydrogenation caused a slower rate and smaller amounts of polyunsaturated fatty acids to reach the small intestine with either intraruminal infusion or dietary feeding. This then influences the pattern of incorporation into the various fractions of the plasma. They also argued that the difference in the two studies was possibly due to different experimental
procedures that were used, where in one study they used relatively long-term intraruminal infusions and in the other they used the single dose infusions of polyunsaturated fatty acids into the abomasum of sheep. Noble et al (142) confirmed the results of Moore et al (130) by intraruminal infusions of emulsions of maize oil or C18:2. However, in their study C18:2 levels began to increase in the plasma triglycerides after 3 hours of infusion. The slower time of appearance of C18:3 in plasma triglycerides compared to the study of Moore et al (130) was probably related to the different places of the infusion (rumen vs abomasum). Noble et al (142) also found that the concentration of C18:2 began to increase in plasma phospholipids after 6-9 hours and cholesterol esters after 24-25 hours. In addition, they found no evidence of absorption mechanisms that lead to direct incorporation of C18:2 into the blood phospholipids or cholesterol esters.

Infusion of maize oil increased the level of C18:0, whereas infusion of C18:2 itself increased C18:1 in plasma triglycerides (142). The variable response was attributed to the difference in the type of fat infused. Maize oil requires hydrolysis of its triglycerides to free fatty acids and then hydrogenation of the released C18:2 into C18:0. Therefore the release of C18:2 from maize triglycerides would be slower than the large dose from C18:2 infusion and the hydrogenation of C18:2 to C18:0 would have more time to be complete. Large doses of C18:2 would saturate the hydrogenation system and the conversion of C18:2 to C18:0 would not be complete. Polan et al (161) indicated that hydrogenation of C18:2 to C18:0 by rumen microorganisms involves two separate systems; one that converts 18:2 into 18:1 and the other that
converts 18:1 into 18:0. They also found that high concentrations of 18:2 completely inhibit the conversion of 18:1 into 18:0 by the second system.

Duncan and Garton (63) showed that grass-fed heifers had in their blood plasma 166, 75, 28, 14, 7 and 1 mg/100ml of cholesterol esters, phospholipids, free cholesterol, triglycerides, free fatty acids and hydrocarbons respectively. Moore et al (132) found that irrespective of dietary treatment 5 or 10% C18:0 or 10% C16:0 fed to lactating cows resulted in 40% of the total plasma fatty acids occurring in cholesterol esters, 54% in phospholipids, 3% in triglycerides and 3% in the NEFA fraction of the blood. The major plasma fatty acid of lactating cows was C18:2 which accounted for about 45% of the total plasma fatty acids. Less than 1% of the total plasma C18:2 was present in the plasma triglycerides (112, 132). Steele et al (188) also found with lactating cows that C18:2 accounted for 46-55% of total plasma fatty acids but only about 1% of this fatty acid occurred in plasma triglycerides.

Following the earlier discovery that formalin treatment prevents microbial degradation of dietary protein in the rumen (71), it has been demonstrated that feeding plant oils rich in polyunsaturated fatty acids coated with formaldehyde-treated casein substantially elevated the polyunsaturated fatty acids in blood plasma of ruminant animals (26, 40, 42, 60, 81, 84, 116, 168, 228). The increase in polyunsaturated fatty acids with the inclusion of formaldehyde-treated oils was in the triglyceride fraction of the plasma (40, 42, 84, 116, 168, 228) and was associated with reductions in concentrations of C16:0 and C18:1 with no change in C18:0 (40, 42).
Formaldehyde-treated plant oils also increased polyunsaturated fatty acids in plasma fractions of cholesterol esters and phospholipids (40, 42, 116, 228) and were associated with reductions in C18:1 and C18:3 fatty acids (42). Yang et al (228) indicated that 35% of the ration of lactating cows fed as protected soybean oil plus sunflower oil increased C18:2 in plasma cholesterol esters and phospholipids. All other fatty acids in these fractions decreased except C18:0 which was slightly increased in plasma phospholipids. Linoleic acid (C18:2) content of plasma NEFA also increased by feeding protected oils (116, 228) and was accompanied by reductions in C16:0 and C18:1 (228). Elevation in polyunsaturated fatty acids of plasma with unprotected plant oils was also reported elsewhere (115, 116). Sharma et al (171) found that feeding dairy cows high levels of protected tallow increased C18:1 and decreased C18:2 with no change in C14:0–C18:0 and C18:3 of the plasma.

Isocaloric replacement of part of the starch in the diet of lactating cows by 8% ground soybeans or soybean oil resulted in increased total plasma fatty acids and the fatty acids found in cholesterol esters and phospholipids (188). Ground soybeans increased concentrations of plasma NEFA and triglyceride fatty acids whereas soybean oil reduced fatty acid concentrations in both plasma fractions. Feeding of ground soybeans increased C18:0 in plasma triglycerides and NEFA increased C18:0 and C18:2 in plasma phospholipids and decreased C18:1 and C20:3 in plasma cholesterol esters. On the other hand, feeding soybean oil resulted in increased concentrations of C18:0 and C18:1 and decreased C18:2 in plasma NEFA. Inclusion of both types
of oils however, caused reductions in C16:0 and C16:1 and increases in C18:2 in plasma cholesterol esters.

The effect of feeding saturated fatty acids on plasma fatty acids was investigated by Moore et al (132). They found that feeding lactating cows 5 or 10% C18:0 resulted in increased C18:0 and decreased C16:0 and C16:1 in plasma triglycerides; increased C18:3 and C20:4 and decreased C16:0 in plasma cholesterol esters. Feeding of 10% C16:0 resulted in increased C16:0 and decreased C18:0, C18:1 and C18:2 in plasma triglycerides; increased C16:1, C18:3 and C20:4 and decreased C18:2 in plasma cholesterol esters (132). Changes in fatty acids composition of plasma phospholipids were also noted (132). Where 5 or 10% C18:0 feeding resulted in increases in C18:0, C18:1 and C18:3 and decreases in C16:0, C18:2, C20:3 and C20:4, 10% C16:0 feeding resulted in increases in C16:0, C16:1, C18:1 and C18:3 and decreases in C18:0, C18:2 and C20:3 fatty acids.

Moody et al (126) found that feeding thermally stressed lactating cows either 6% tallow or 5.5% tallow plus 0.5% sucroglyceride caused no change in the plasma fatty acids of cholesterol esters, total lipids, triglycerides, free fatty acids, mono and diglycerides or phospholipids. However, an environmental temperature of 32.9°C resulted in increases in C10-C15 fatty acids in both plasma triglycerides and free fatty acids.
Influence of Dietary Fat on Milk Production and Milk Composition

Milk Yield

Inconsistent results were found in the literature regarding the influence of dietary fats and oils on the amounts of milk produced by lactating cows. The inconsistency of the results however, could be attributed to several factors which must be considered when attempting comparisons. Type of fat (animal, plant or marine), level of fat and level and type of roughage in the diet are the main factors involved. Other influencing factors could be breed of animal, composition of ration, stage of lactation and possibly level of production.

As early as 1932 Petersen (158) reported that cod liver oil had no effect on the amount of milk produced by different breeds of lactating dairy cows. Cod liver oil was added in amounts of 5 ounces per day to a grain mixture of corn, barley, oats and cottonseed meal added to a roughage diet of alfalfa hay and corn silage. Cod liver oil also produced no significant change in the amounts of milk produced in other studies (18, 75). Intravenous infusion of 400 g/day of cod liver oil or ethive (an ethyl ester fraction of cod liver oil rich in polyunsaturated acids) emulsions tended to depress milk yield (198). The depressing effect of milk yield with cod liver oil and ethive infusion however, was only produced during the first milking on the first day and was attributed to the pyrogenic reaction to the emulsions. Conversely intravenous infusion of 500 g/day of soybean oil emulsion for 2 days had no effect on daily milk yield (198). Storry et al (200) also investigated the infusion of nine artificial emulsions of synthetic triglycerides into the jugular vein of lactating cows for 2
days. The infused triglycerides ranged from tripropionine to triolein. Both cows infused with tributyrin and one cow from each of the two cows infused with tricaprylin and tricaprin showed significant reductions in milk yield. The other synthetic triglycerides resulted in no change in milk yield.

Various animal fats and vegetable oils fed to milking cows at various levels failed to result in marked changes in milk yield (2, 35, 51, 75, 88, 104, 111, 123, 124, 126, 145, 156, 169, 176, 181, 184, 199, 205). Allen (2) in a series of short-term experiments found that butter fat, lards, tallow, linseed oil, cottonseed oil, corn oil, peanut oil, and soybean oil slightly influenced milk yield. However coconut oil caused a depression in milk yield when fed at the level of 1.25 pounds/day. Coconut oil added to a basal diet at 0, 2, 4, 7 and 10% also showed significant (p<0.05) reduction in milk yield but only at the highest level of oil and only during the first 12 days of the experiment (196). No significant change in milk yield during the last 12 days of supplementation was noticed. Other studies involving the use of coconut oil showed no change in milk yield (123, 199).

The effect of adding various fats and oils to rations was also tested in a series of short (5 day), medium (10 day) and long (20 day) term experiments (75). It was found that soybean oil, cod liver oil, and butter had no effect on milk yield whereas, cottonseed oil tended to increase milk yield. Others (35, 181, 184) reported no effects of cottonseed oil on milk yield. Any effect noted was related to the level of the roughage in the diet. The effects of feeding whole cottonseed on milk yields were inconsistent. Where some (51, 176)
found no effects, Anderson et al (4) found that whole unprocessed cottonseed significantly increased total milk production.

Method of incorporation of the oil into the diet has been shown to effect milk yield (221). Williams et al (221) showed that feeding cracked soybeans decreased milk yield, whereas, feeding soybean oil resulted in an increased milk yield of 7.8 pounds/cow/week. Other studies (2, 75, 104, 111, 124, 156) reported no change in milk yield due to the feeding of ground soybeans or soybean oil. Conversely, Steele et al (187) indicated that substitution of part of the starch in the concentrate mixture with 8% soybean oil or ground soybeans resulted in significant increases in milk production. Banks et al (14) also found that diets supplemented with soybean oil, palm oil/palmatic acid mixtures or tallow increased milk production by 24-45% over a basal diet low in fat. A basal diet supplemented with ground nuts significantly increased milk yield, whereas supplements of coconut oil or red palm oil failed to result in significant changes in milk yield (199). By contrast, Storry et al (195) reported increases in milk yield when a concentrate mixture was supplemented with 2, 4 and 8% red palm oil.

Influence of roughage level in diets supplemented with fat has been under investigation (35, 181, 184). Brown et al (35) indicated that low-roughage diets supplemented with 6% cottonseed oil significantly reduced milk yield. Low-roughage diets supplemented with 6% tallow however, failed to show such response (35). Addition of 6% tallow or cottonseed oil to high-roughage diets also had no effect on total milk yield (35). In addition, low-roughage diets regardless of
the dietary fat feeding produced more milk compared to high-roughage diets (35). Conversely, Steele and Moore (181) found that changing from a high-roughage diet with no fat to a low-roughage diet also with no fat had no effect on milk yield. They did find that low-roughage diets fed with a concentrate mixture containing 10% cottonseed oil significantly increased milk yield compared to high-roughage diets fed with the same level of cottonseed oil. Nevertheless, the same authors (181) in another experiment found that the addition of 5 or 10% cottonseed oil to concentrate mixtures and fed with high-or low-roughage diets produced no significant changes in milk production. Similarly, Steele and Moore (184) showed that 6% tallow or cottonseed oil added to low-roughage or high-roughage diets resulted in no significant change in milk yield. Concentrate mixtures containing 10% tallow or cottonseed oil added to high-roughage diets also did not effect milk yield. Addition of the same mixtures to low-roughage diets resulted in a small but significant improvement in milk yield (184).

Supplementation of grain mixtures with fatty acids were studied by British workers (180, 183, 185). The addition of 5% oleic acid (C18:1) significantly increased milk yield whereas 10% C18:1 or 10% of a mixture of saturated fatty acids (C16:0 + C18:0) had no effect (185). Steele and Moore (183) indicated that isocaloric replacement of part of the concentrate mixture with 10% myristic acid (C14:0) significantly reduced milk production whereas, 5% lauric acid (C12:0) or 10% palmitic (C16:0) or stearic acids (C18:0) had no significant effects. Conversely, Steele (180) reported a significant increase in
milk yield from the inclusion of 5 or 10% C16:0 or C18:0 acid in the concentrate diet of lactating cows.

Increased milk yield with tallow feeding has been reported in the literature (1, 14, 201). Adams et al (1) indicated that 10% tallow or vegetable oil significantly increased milk yield, possibly due to the increase in energy intake. Concentrate mixtures with 7% beef tallow also increased milk production significantly (201). By contrast, Palmquist and Conrade (146) showed that a 10% tallow diet compared to either a no fat diet or a diet with 10% blended animal-vegetable fat produced a low milk yield. The reduction in milk yields at high levels of tallow feeding was not clear but it was suggested that lower feed intake and increased body weights of the cows on high tallow feeding were responsible for this reduction. Others (88, 126) reported no change in milk yield due to tallow feeding.

A significant depression in milk yield was reported by feeding 15% safflower oil (154). Rafalowski and Park (162) on the other hand found that a 10% whole sunflower seed diet increased milk production. Increasing the level of sunflower seed to 20 or 30% failed to show a response (162).

Considerable research has been directed toward incorporating formaldehyde-treated unsaturated plant oils and animal fats in the ration of lactating dairy cows. The majority of these works resulted in either no change (15, 64, 81, 83, 113, 149, 160, 171, 177, 193, 222, 226) or in an increase in milk production (117, 224). Formaldehyde-protected and unprotected safflower oil-casein fed at 1500 g/day produced no significant change in milk production when compared to a basal
diet of no added oil (160). Similar results were obtained by inclusion of protected and unprotected cod liver oil (193), safflower oil (81) and soybean and cottonseed oil (83). Macleod et al (113) studied the effects of feeding high levels of protected-tallow on the performance of dairy cows. They found that actual milk yield was not affected by feeding up to 32% of the diet as protected-tallow. However, 4% fat-corrected-milk (FCM) was significantly increased due to the increase in milk fat test. The results of these Canadian workers were supported by others (15, 64, 149, 171, 177, 222). Significant improvement in milk yield was noticed when Jersey cows were supplemented with either 3.6 kg/day full-fat soyflour or 3.6 kg/day protected full-fat soyflour (117). The supplements were compared with a control diet of corn silage, alfalfa hay, and grain concentrate. Increase in digestible energy intake was explained to be the reason for the increase in milk production. Moreover, Wrenn et al (224) reported 8 to 10% increase in milk yield when feeding protected or unprotected tallow. Protected soybean-tallow fed at 0, 1.7, 3.0 or 5.0 kg/day with a hay/concentrate diet resulted in increased milk yield at low levels of fat but reduced yields at the highest level of protected supplement (24). Reduction in milk yield with untreated coconut oil compared with diets of either no oil or protected coconut oil was reported by Storry et al (191). More recently, Murphy and Morgan (134) found that inclusion of unprotected tallow in the concentrate part of the diet resulted in no significant change in milk yield whereas, inclusion of a protected tallow supplement increased milk yield significantly. They concluded that the poor response to the untreated tallow was possibly related
to the reduction in the digestibility of the ration, particularly that of fiber, and to the reduction in roughage intake.

**Milk Fat and Fatty Acids**

**General Considerations.** Over the last 60 years considerable attention has been given to the effects of dietary lipids on milk production and on milk composition particularly milk fat and milk fatty acids. The composition, biosynthesis and secretion of milk fat have been extensively reviewed by both Garton (78) and Storry (189). Milk fat depression syndrome and the use of dietary fat in dairy rations has also been reviewed by Van Soest (210) and Palmquist and Jenkins (147).

Generally, dietary saturated fats tend to maintain or increase milk fat (3, 37, 75, 88, 111, 126, 141, 180, 183, 191, 197), whereas unsaturated oils tend to depress it (3, 37, 75, 111, 133, 154, 158, 172, 185, 196). Method of incorporation of the dietary lipid, level of dietary lipid, frequency of feeding, level of the roughage in the diet and level of the lipid in the basal diet are factors to be considered (189).

Cracked soybeans maintained or even increased milk fat production whereas, equivalent amounts of soybean oil depressed it (104, 108, 187, 221). Moore et al (133) indicated that feeding milking cows 5-8 ounces of cod liver oil in one feeding each day caused a pronounced decrease in milk fat. When the same quantity of oil was fed in 12 equal feedings each day there was no depression in fat content. Low milk fat was produced from diets low in roughage and high in concentrates (10, 11, 12, 50, 181). Low-roughage diets supplemented with
5 to 10% cottonseed oil failed to restore the depression in milk fat content or yield (35, 181, 184). However, inclusion of cottonseed oil or tallow with high-roughage diets resulted either in no change or an increase in milk fat (35, 184). The response to dietary fat is small possibly due to the fact that most basal diets of lactating cows contain normal amounts of fat (199). Low-fat basal diets supplemented with fat produced increases in milk fat percentage or milk fat yield (14, 180, 213). Storry et al (196) found that when basal low-fat diets were supplemented with 2, 4, 7 or 10% coconut oil, milk fat yield was depressed only on the highest level of supplementation.

Davis and Harland (51) reported that when 0.9 kg of concentrate mixture was replaced by cottonseed and fed to lactating cows along with a high-roughage diet, an increase in milk fat yield was noticed during the first 20 days of the experiment. However, this response was not maintained beyond the 20 days. Similar observations were noticed by Steele and Moore (184) with cottonseed oil and Storry et al (195) with red palm oil. This may reflect an adaptive change in digestion or milk fat synthesis by the animal (46).

**Origin of Milk Fatty Acids.** To fully understand the mechanism by which dietary lipids negatively or positively influence milk fat we must consider its effect on the individual fatty acids of milk fat. Milk fatty acids can be categorized into three types according to their origin, namely; short-chain fatty acids (C4-C10), intermediate-chain fatty acids (C12-C16) and long-chain fatty acids (C18) (189). Fatty acids of chain length up to C14 and part of C16 are known to be synthesized by de novo synthesis in the mammary gland from acetate and
BHBA (6, 91, 102, 107, 151, 155). James et al (91) with perfused bovine mammary glands showed that short and intermediate chain fatty acids of milk fat are elongated by successive addition of 2-carbon units, and that odd-numbered fatty acids of milk fat are synthesized by condensation of acetate with propionate. Part of C16 and possibly all of C18 are derived from sources other than de novo synthesis in the mammary gland (6, 21, 107, 151, 155). Bishop et al (25) and Palmquist et al (151) indicated that 30-40% of C16 come from de novo synthesis in the mammary gland. Long-chain fatty acids on the other hand, are taken up by the mammary tissue from plasma triglycerides of chylomicrons and low-density lipoproteins (6, 16). These plasma triglycerides are then partially or completely hydrolyzed and the resulting fatty acids are incorporated into new triglyceride molecules in the mammary gland (16, 155). Annison et al (6) indicated that 60% of milk fat come from plasma triglycerides. Conversely, plasma phospholipids, sterols and sterol esters and their fatty acids do not contribute fatty acids to milk fat (6). NEFA are in equilibrium with a pool of free fatty acids in the mammary gland (6, 16, 218, 219). Palmquist and Mattos (148) showed that 76% of the absorbed lipid is taken up by the mammary gland and that 44% of milk fat is of direct dietary origin. Since Garton (78) indicated that 50% of milk fat is from long-chain fatty acids, it follows that 6% of milk fat comes from endogenous origin (148).

The influence of dietary fat on milk fat secretion would therefore depend on the balance between the synthesis of short- and intermediate-chain fatty acids and the absorption of long-chain fatty acids.
from the plasma triglycerides. Dairy rations which are known to reduce milk fat are those rations which depress synthesis of short-and intermediate-chain fatty acids with little or no increase in long-chain fatty acid absorption from plasma triglycerides. Such diets are those with low levels of roughage and no dietary fat supplement (10, 11, 12, 211) or diets with high levels of unprotected polyunsaturated fatty acids (3, 14, 18, 37, 75, 108, 133, 154, 158, 172, 181, 184, 185, 187, 191, 193, 196, 221). On the other hand, dairy rations which tend to maintain or increase milk fat are those rations with high levels of roughage (35, 141, 145, 180, 183, 184), high levels of saturated fatty acids (3, 14, 37, 88, 111, 126, 185, 197, 201) or formaldehyde-protected lipids (8, 15, 64, 84, 101, 113, 134, 152, 160, 171, 191, 192, 193, 194, 222, 224, 225, 226).

Milk Fat Depression. Several explanations were provided regarding the depression in milk fat of lactating cows. Some of these explanations were reviewed by Van Soest (210). Nevertheless, influence of dietary fat will be the main subject in this discussion. One effect is the change in rumen fermentation by increasing ruminal propionate and decreasing ruminal acetate. Since acetic acid is known as a precursor for milk fatty acids synthesis of chain-length C4-C16 (6, 91, 102, 107, 151, 155) any reduction in ruminal acetate would result in a reduction in the rate of synthesis of these fatty acids in the mammary gland and hence a reduction in milk fat secretion. Shaw et al (174) found a positive correlation (+ 0.64) between milk fat percentages and rumen acetate and a negative correlation (- 0.63) between milk fat percentages and rumen propionate. Balch and Rowland (10)
indicated that 0.5-1.5 kg of sodium acetate administration tended to restore the content of milk fat that was depressed by the feeding of a high-grain restricted-roughage diet. Sodium propionate administration at 414 g/day failed to restore milk fat content which was depressed by low hay diets. The relationship between dietary fats and rumen VFA was previously discussed in the section on the effect of dietary lipid on rumen VFA and the reader may refer to that. Brumby et al (37) studied the effect of feeding 300 g/day of native cod liver oil, moderately hydrogenated cod liver oil, and severely hydrogenated cod liver oil. They found that native cod liver oil resulted in a significant reduction in both milk fat content and yield, reduced mammary gland synthesis of fatty acids and reduced fatty acid uptake by the mammary gland from plasma triglycerides. None of these effects resulted from the feeding of the moderately or severely hydrogenated cod liver oils. In addition, all types of oils resulted in increased secretion of C20-C22, however only native cod liver oil resulted in low rumen acetate and high rumen propionate. They concluded that the effect of cod liver oil on milk fat was through its effect on rumen fermentation and not from the effect of C20-C22 fatty acids of cod liver oil. The study of Brumby et al (37) was supported by the work of Storry et al (193) and Storry et al (191) with formaldehyde-protected cod liver oil and coconut oil respectively.

Another effect was suggested by Van Soest and Allen (211). They indicated that rather than being due to reduced rumen acetate the reduction of milk fat was partially due to an increase in rumen propionate which acts as an antiketogenic factor by reducing plasma
ketone bodies. This was previously mentioned in the VFA section of the literature.

Reduction in rumen acetate by dietary fat has not been confirmed in other studies involving reduction of milk fat secretion (8, 18, 185, 197). This would lead us to a third effect in the depression of milk fat which was suggested by Moore and Steele (128). These authors indicated that increased uptake by the mammary gland of long-chain fatty acids from plasma triglycerides (which accompanied the feeding of dietary fats) leads to increased concentrations of long-chain fatty acids and their fatty acyl CoA derivatives in the mammary gland. Increased concentrations of fatty acids or fatty acyl CoA derivatives inhibit acetyl CoA carboxylase (86, 89, 178) which catalyzes the rate limiting step in fatty acids synthesis in the bovine mammary gland (74).

It has been indicated that ingested polyunsaturated fatty acids are extensively hydrogenated in the rumen (5, 22, 79, 207). Ward et al (216) showed that trans 18:1 fatty acid was an intermediate in the biohydrogenation of C18 polyunsaturated fatty acids by rumen microorganisms. It has also been shown by Bickerstaffe et al (22) that cis-and trans-isomers of fatty acids are equally metabolized, absorbed from the small intestine, transferred into the lymph, taken up by the mammary gland and incorporated into milk fat. Moreover, increases in trans C18:1 of milk fat as a result of increased ingestion of polyunsaturated fatty acids has been reported in the literature (13, 176, 181, 186). Smith et al (176) indicated a fourfold increase in milk trans C18:1 by feeding high levels of whole cottonseeds. It has been
suggested (49) that trans-acids which are formed during the hydrogenation of polyunsaturated fatty acids in the rumen may exert an inhibitory effect on the synthesis of fatty acids in the mammary gland. Rindsig and Schultz (165) investigated this effect by infusion of safflower oil or elaidic acid (trans-9-octadecenoic acid) into the abomasum of lactating cows. Infusion of 250 ml of safflower oil over 4 hours daily for 3 weeks increased milk fat percentage and increased concentrations of C18 polyunsaturated fatty acids of milk fat, but decreased concentrations of C14:0, C16:0, C18:0 and C18:1. In another trial, they found no effect on milk fat yield or percentage from infusion of 500 ml of safflower oil over a daily 4 hour period for one week. The infusion did increase the concentration of polyunsaturated C18 fatty acids and decreased the concentrations of C14:0, C:16:0, and C18:0. In a third trial, infusion of 25 grams daily of elaidic acid did not effect milk fat yield or percentage. As a result, it was concluded that the depression in milk fat by feeding polyunsaturated fatty acids was possibly due to other rumen metabolites but not to the trans-fatty acids. The results of Rindsig and Schultz (165) were also confirmed by Bickerstaffe et al (22). Selner and Schultz (169) however, were able to confirm Davis and Brown's (49) hypothesis but they did not exclude the possibility of the formation of other products during the production of trans-acids which could inhibit fatty acids synthesis in the mammary gland. In their study (169) they found that feeding 250 or 500 ml per cow per day of C18:1 did not reduce milk fat content but trans-18:1 fatty acid was increased in the milk fat. Conversely, feeding hydrogenated vegetable oil containing 13%
trans-acids at 454 grams/cow/day slightly reduced milk fat content and increased trans-18:1. Feeding oil high in trans-18:1 acid (49%) at 454 grams/cow/day however, decreased milk fat content and decreased milk fatty acids from C8 to C14 and partially reduced C16. C18:1, C18:2 and C18:3 were increased. High trans-acid oil feeding also increased trans-18:1 in milk fat.

Yet another explanation for the reduction in milk fat in association with dietary fat is through the inhibition of lipoprotein lipase of the mammary gland. The presence of lipoprotein lipase in the mammary gland of goats (16) and guinea pigs (121) has been observed. This enzyme is responsible for the hydrolysis of the plasma triglycerides which is the first step in the uptake of the fatty acids by the mammary gland (219). Increases in the secretion of C20-C22 fatty acids with the feeding of protected or unprotected cod liver oil (37, 193) or by intravenous infusion of cod liver oil (198) were observed. Storry et al (198) found that intravenous infusion of cod liver oil increased plasma C20-C22 fatty acids but reduced total milk fat yield. The reduction in milk fat yield was due to the reduction in the C14-C18 acids with no apparent effect on the C4-C12 acids. They postulated that cod liver oil reduces milk fat yield in a way which involves two mechanisms. If cod liver oil is fed or infused intraruminally it may change the fermentation process in the rumen which may effect the synthesis of short-chain fatty acids. However, if cod liver oil is infused intravenously or if some of the C20-C22 escapes hydrogenation, these long-chain fatty acids will then inhibit the secretion of the long-chain fatty acids of milk fat through inhibition of
lipoprotein lipase. This hypothesis, however, was not confirmed in the studies of Brumby et al (37) or Storry et al (193).

**Milk Fatty Acids.** Short and Intermediate Chain Fatty Acids (C4-C16): As previously mentioned these fatty acids are derived mainly from de novo synthesis in the mammary gland from acetate and BHBA (6, 91, 102, 107, 151, 155). Intermediate-chain (C12-C16) fatty acids may also be partly derived from plasma triglycerides since increased dietary intake tended to increase their concentrations in milk fat (123, 183, 199). General reductions in the secretion of short or intermediate chain fatty acids were found with the dietary inclusion of free lipids (13, 35, 87, 112, 127, 141, 154, 162, 169, 176, 177, 181, 183, 184, 185, 186, 195, 197, 205) or formaldehyde-protected lipids (15, 64, 84, 117, 160, 171, 193, 194, 228). The reduction in the de novo synthesis of milk fatty acids was calculated to be 40-50% (87, 176, 177, 205). Storry (189) reviewed two mechanisms which are related to the reduction in the secretion of short and intermediate chain fatty acids of milk fat with the inclusion of dietary lipids. The first mechanism discussed was the change in rumen fermentation associated with changing molar percentages of the VFA which lead to reduced availability of acetate and BHBA for the mammary gland. The other one was the increased uptake of long-chain fatty acids by the mammary gland from plasma triglycerides normally associated with dietary feeding of lipids. This mechanism reduces mammary gland synthesis of fatty acids through inhibition of acetyl CoA carboxylase. These two and other mechanisms were previously discussed under the section of milk fat depression.
The effect of dietary lipids on C16 fatty acids was variable. In some cases it increased it (13, 141, 154, 183, 185, 192, 195) and in others decreased it (13, 15, 42, 84, 117, 127, 141, 156, 160, 162, 171, 181, 183, 184, 185, 186, 193, 194, 199, 226, 228). The variability of the C16 response to dietary lipids was possibly related to the fact that C16 fatty acids are partially synthesized in the mammary gland (25, 151) and partially taken up from plasma triglycerides. Also de novo synthesis of C16 fatty acids is depressed as a result of the addition of dietary fat. Net yield of C16:0 may depend upon the balance between the amount derived from intramammary synthesis and the amount taken up from plasma triglycerides (189). Steele and Moore (185) indicated that increased uptake of C18 with reduced uptake of C16:0 such as in the feeding of red palm oil, tallow or cottonseed oil, will cause either no change or a reduction in C16. However, it is possible to increase the C16:0 in milk by feeding large quantities of C16:0 and less of the C18 acids. Palmitoleic acid (C16:1) however, was shown to be synthesized from C16:0 in the mammary gland (25). Butyric acid (C4:0) unlike other short and intermediate chain fatty acids of milk fat may not be affected by dietary fat (64, 228) or it may actually increase during lipid feeding (15, 64, 117, 141, 186, 195, 196, 197, 228). This could be explained by the work of Kumar et al (102). In experiments with rabbit mammary glands they showed that avidin markedly inhibited the synthesis of C6-C16 fatty acids when acetate, acetoacetate, butyrate or BHBA were used as substrates. Conversely, avidin had little effect on the synthesis of butyrate when the same substrates were used. This indicates that the synthesis
of C4:0 does not involve the carboxylation of acetyl CoA and that the increase in the uptake of long-chain fatty acids which inhibit acetyl CoA carboxylase will reduce C6-C16 but not C4:0.

Long-Chain Fatty Acids (>C16): Long-Chain fatty acids are derived from plasma triglycerides of chylomicrons and low density lipoproteins (6, 16). Dietary feeding of polyunsaturated fats and oils resulted in marked increases in the output of C18:0 and C18:1 but not C18:2 or C18:3 (64, 87, 112, 154, 162, 171, 176, 181, 184, 186, 194, 207). This is a reflection of the extensive hydrogenation of these polyunsaturated fatty acids in the rumen since bypassing the rumen by intravenous infusion (190, 198, 207) or feeding of formaldehyde-protected fats (27, 42, 83, 84, 117, 160, 192, 225, 226, 227, 228) significantly increased their concentrations in milk fat. Linoleic acid and C18:3 may also be derived from dietary origin by escaping hydrogenation in the rumen. This is especially true with the feeding of raw ground plant seeds rich in these fatty acids (108, 221). Stearic acid (C18:0) is derived from direct dietary origin such as feeding of pure C18:0 or animal tallow (13, 141, 183, 184, 192, 197) or from biohydrogenation of polyunsaturated fatty acids in the rumen (22, 112, 127, 176, 184, 186, 195, 207). Oleic acid on the other hand, is derived from more than one pathway. It could be from direct dietary origin such as the feeding of C18:1 or tallow (13, 64, 171, 184, 185, 192, 194, 197, 201) or from incomplete hydrogenation of dietary polyunsaturated fatty acids in the rumen (13, 18, 83, 87, 112, 127, 154, 160, 162, 176, 181, 184, 186, 195, 207, 224). Incomplete hydrogenation of polyunsaturated fatty acids will result in increased
trans-C18:1 in milk fat (13, 176, 181, 186, 216). Oleic acid may also be derived from the desaturation of C18:0 in the epithelial cells of the small intestine after absorption (20, 22) or from desaturation of C18:0 in the mammary gland (19, 21, 41, 105). However, intestinal or mammary gland desaturation will also increase Cis-C18:1 in milk fat (41, 141, 183).

The possibility of de novo synthesis of C18:0 and C18:1 in tissue other than the mammary gland has not been excluded (80, 186). Steele et al (186) calculated the daily intake of the C18 acids from a control diet with no added dietary fat and found it to be 67.9 g/day. The daily output of these acids was 98.0 g/day. Since there were no changes in body weights of the cows and the C18 acids were not synthesized in the mammary gland, they concluded that the 30.1 g difference probably was derived from either de novo synthesis in tissues other than the mammary gland or by rumen microorganisms.

Other long-chain fatty acids such as C20-C22 are apparently of direct dietary origin (37, 87, 193).

Other Milk Components

Influence of dietary lipids on milk protein and solids-not-fat (SNF) was found to be variable. Changes in milk components by inclusion of dietary fats is possibly related to the effect on total milk yield. Nevertheless, milk protein was not shown to be affected by dietary fat feeding by some researchers (15, 35, 83, 104, 123, 126, 146, 156, 162, 224, 226). By comparison, others reported reductions in milk protein content by the feeding of free fats (14, 111, 176, 180) or protected fats (64, 113, 117, 134, 149, 171, 191). Dunkley et al (64)
indicated that the decrease in protein content of milk with the feeding of protected fat was in the casein fraction and that the amino acid composition of milk protein was not affected. The reduction in milk protein content with inclusion of formaldehyde-protected fat could be attributed to overprotection of the protein-fat complex which may lower the degradability of both (59, 117). However, Dunkley et al (64) and Smith et al (177) suggested that the presence of high amounts of protected lipid in the ration might reduce carbohydrate availability to the microorganisms of the rumen and consequently depress microbial protein synthesis causing the reduction in milk protein. In addition, Palmquist and Moser (149) related the depression in milk protein from the inclusion of protected lipid to plasma glucose and insulin levels. They found that protected fat tended to depress milk protein but increased milk fat percentage. Plasma glucose and insulin were also reduced by the feeding of the protected supplement. It was suggested that dietary fat may impair amino acid transport to the mammary gland which may reduce milk protein secretion by inducing insulin resistance.

In a review of dietary effects on milk protein secretion, Emery (66) suggested carbohydrates or other materials capable of increasing blood glucose must be increased in the diet in order to increase milk protein secretion.

Protected-fat ingestion may also increase milk protein (8, 152). Astrup et al (8) showed that protected soybean oil increased milk protein whereas, protected-hydrogenated soybean oil did not suggesting that secretion of milk protein may be related to the degree of unsaturation of dietary fat.
Depression of the SNF content of milk with dietary feeding of fat has been shown by many workers (64, 152, 176, 177, 180, 184, 187, 191, 224). Conversely, Goering et al (83) reported significant increases in SNF content and yield by feeding protected or unprotected soybean oil with no change in total milk production. Others (1, 14, 104, 126, 160, 171, 181) however, reported no change in milk SNF by the inclusion of fat in the diet of lactating dairy cows.
EXPERIMENTAL PROCEDURE

A control ration containing a 50:50 ratio of concentrate to alfalfa hay was used to compare the effects of adding calcium treated animal fat to dairy rations. The concentrate portion was a commercially available mixture consisting mainly of corn, barley, and a formulated supplement. In addition to the control ration four other experimental rations were fed: 1) control with 6% calcium treated animal fat (4%TF), 2) control with 12% calcium treated animal fat (8%TF), 3) control with 4% animal fat (4%F), 4) control with 8% animal fat (8%F). The 6 and 12% levels of calcium treated animal fat were added to cause the inclusion of 4 and 8% animal fat in the respective rations. In all cases the added ingredients replaced an equal weight of the concentrate mixture and were mixed with the concentrates prior to feeding. Cottonseed meal (10%) and molasses (4%) were also added to all experimental mixtures. Tables 1 and 2 show actual ration composition and chemical analysis.

Twenty-five Holstein cows from the University of Arizona dairy were selected for nearness to peak of lactation and randomly assigned to one of the five treatments. The experiment consisted of a single six week period from June 15 through July 27, 1983. Two days before feeding the experimental diets, milk and blood samples were taken for pretreatment analysis. Individual body weights of all cows were determined. Cows were penned by treatment and group-fed twice daily, approximately 110% of their requirements for maintenance and production.
Table 1. Composition of Experimental Diets.

<table>
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<tr>
<th></th>
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<th>4%TF</th>
<th>8%TF</th>
<th>4%F</th>
<th>8%F</th>
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<td>Alfalfa hay (%)</td>
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<td>50</td>
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<td>Commercial concentrate mixture (%)</td>
<td>36</td>
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<td>24</td>
<td>32</td>
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<td>Cottonseed meal (%)</td>
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<td>Molasses (%)</td>
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<td>Calcium treated animal fat</td>
<td>-</td>
<td>6</td>
<td>12</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Animal fat (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Alfalfa</td>
<td>Control</td>
<td>4%TF</td>
<td>8%TF</td>
<td>4%F</td>
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<td><strong>Dry matter (%)</strong></td>
<td>92.00</td>
<td>89.26</td>
<td>88.58</td>
<td>88.19</td>
<td>88.80</td>
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<td><strong>Ash (%)</strong></td>
<td>11.13</td>
<td>5.05</td>
<td>6.37</td>
<td>7.56</td>
<td>4.67</td>
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<td><strong>NDF</strong>&lt;sup&gt;1&lt;/sup&gt; (%)</td>
<td>42.46</td>
<td>25.66</td>
<td>23.43</td>
<td>22.76</td>
<td>24.42</td>
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<td><strong>Cell-Solubles (%)</strong></td>
<td>57.54</td>
<td>74.34</td>
<td>76.57</td>
<td>77.24</td>
<td>75.58</td>
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<tr>
<td><strong>ADF</strong>&lt;sup&gt;2&lt;/sup&gt; (%)</td>
<td>34.94</td>
<td>10.16</td>
<td>8.89</td>
<td>8.17</td>
<td>9.10</td>
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<tr>
<td><strong>Cellulose (%)</strong></td>
<td>26.19</td>
<td>7.01</td>
<td>6.49</td>
<td>5.9</td>
<td>6.6</td>
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<td><strong>Hemicellulose (%)</strong></td>
<td>7.52</td>
<td>15.5</td>
<td>14.54</td>
<td>14.59</td>
<td>15.32</td>
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<tr>
<td><strong>Lignin (%)</strong></td>
<td>8.24</td>
<td>2.01</td>
<td>1.63</td>
<td>1.47</td>
<td>1.52</td>
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<td><strong>Ether Extract (%)</strong></td>
<td>2.32</td>
<td>2.81</td>
<td>6.23</td>
<td>10.01</td>
<td>6.75</td>
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<td><strong>CM</strong>&lt;sup&gt;3&lt;/sup&gt; (%)</td>
<td>3.85</td>
<td>4.24</td>
<td>7.78</td>
<td>9.74</td>
<td>8.29</td>
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<td><strong>CMH</strong>&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>10.60</td>
<td>5.32</td>
<td>8.74</td>
<td>12.35</td>
<td>8.68</td>
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<tr>
<td><strong>Crude Protein (%)</strong></td>
<td>19.56</td>
<td>19.02</td>
<td>18.37</td>
<td>14.96</td>
<td>17.32</td>
</tr>
<tr>
<td><strong>Calcium (%)</strong></td>
<td>1.90</td>
<td>0.70</td>
<td>0.79</td>
<td>1.14</td>
<td>0.72</td>
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<tr>
<td><strong>Phosphorus (%)</strong></td>
<td>0.32</td>
<td>0.67</td>
<td>0.57</td>
<td>0.60</td>
<td>0.68</td>
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<tr>
<td><strong>Combustible Energy (Kcal/g)</strong></td>
<td>4.30</td>
<td>4.43</td>
<td>4.52</td>
<td>4.70</td>
<td>4.63</td>
</tr>
</tbody>
</table>

<sup>1</sup>Neutral-detergent fibers; <sup>2</sup>Acid-detergent fiber; <sup>3</sup>Chloroform-Methanol (2:1 Vol.); <sup>4</sup>Chloroform-Methanol-Hydrochloric acid (60:40:1 Vol.)
(135). By the end of the third week of the experiment, the cows were fed the concentrate mixture individually but continued to be group-fed alfalfa hay. It was assumed that the cows consumed the roughage proportionally according to their body weights. The cows were fed at approximately 0630 and 1830 h and were milked at 0500 and 1700 h.

Milk samples collected during four consecutive milkings at the end of each week were composited and analyzed within 48 hours for each cow. Milk fat was determined by the standard Babcock method, protein by the Orange G method of Udy (208), and Solids-Not-Fat (SNF) by Watson (217). The weights of all milkings were recorded and average daily milk productions for each week were determined. These figures for each week were averaged to determine the average daily production for the entire period. Percent fat, percent protein, and percent SNF were determined in the same manner.

Fat samples from the milk of each cow from the pretrial collection and the third and sixth week were frozen at -10°C until analyzed by gas-liquid chromatography for component fatty acids. In preparation for gas-liquid chromatography, approximately 0.2 cc of fat was separated from the milk by the TeSa fat test method and dried under vacuum. The fatty acids were converted to their methyl esters by transmethylation using sodium methoxide (109). The methyl esters were extracted in hexane and separated using a Perkin-Elmer Model 800 dual flame gas-liquid chromatograph. The 2m X 2mm glass column was packed with 10% DEGS-PS on 80/100 Supel coport and had a programmed temperature of 90-200°C, flow rate of 40ml/min., inlet temperature of 240°C and a detector temperature of 220°C. Peaks and weight percentages of
individual fatty acids were quantitated using a Hewlett-Packard Model 3392A digital integrator.

Blood samples from the tail vein were collected for the pre-trial, third and sixth week periods of the trial. Blood plasmas were separated by centrifugation and frozen for analysis at -10°C. Total serum lipids were extracted using the procedure described by Folch et al (73). Serum total cholesterol were determined colorimetrically by a commercially prepared kit (SIGMA Technical bulletin No. 350) and a Perkin-Elmer, Lambda I-UV/Vis spectrophotometer. Fatty acids were determined using serum total lipid extracts. Methyl esters of the fatty acids were detected and identified in the same manner as the milk fatty acids except an isothermally fixed temperature of 160°C was used.

Fecal samples were collected by rectal removal twice daily (0700 and 1900) during six consecutive days at the end of the experiment and were composited for each cow. Dry composite feces (dried at <50°C), alfalfa hay and each concentrate mixture were analyzed for dry matter, ether extract, ash, crude protein, phosphorus, and calcium percentages according to A.O.A.C. methods (7). Cell wall constituents were determined as described by Goering and Van Soest (82). Gross energy was measured with an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

Lipids were extracted from both the feed and feces using chloroform-methanol (2:1 Vol.) and chloroform-methanol-hydrochloric acid (60:40:1 Vol.) as described by Marchello et al (114). Fecal soaps were determined by gravimetrical difference between the chloroform-methanol-hydrochloric acid extract and the chloroform-methanol extract
as outlined by Figroid (72). Fatty acid composition of extracted lipid samples was determined as previously described with milk fat and serum total lipid.

Rumin fluid samples were taken by rumen tube on the morning of the last day of the experiment. The rumin fluid samples were strained through a layer of cheese cloth and frozen at -10°C until analyzed by the method of Erwin et al (68) using a Tractor MT 220 gas liquid chromatograph and the previous described digital integrator. The glass column of the chromatograph was packed with 10% SP - 1000/1% H3PO4 on 100/200 chromosorb WAW with a flow rate of 30 ml/min, isothermal temperature of 140°C, inlet temperature of 193°C, and a detector temperature of 185°C.

Body weights were measured once two days before the feeding trial and three times on the consecutive last three days of the experiment. The last three figures were overaged to determine final body weights.

Calcium treated animal fat was prepared using a modified double decomposition procedure described by Palmquist (personal communication). Animal fat (18.2 kg) was melted and heated to ca 90°C using a double layer stainless steel steam heating vat. Crystal NaOH (3.5 kg) was dissolved in cold water and added to the preheated animal fat and mixed for about 30 minutes while maintaining the temperature at 90°C after which 4.0 kg of dry CaCl2 were dissolved in cold water and added to the solution of animal fat and NaOH. The formed calcium soaps of tallow were then sun dried, pulverized by hand and mixed with the other concentrate ingredients.
Apparent digestibilities were estimated using the lignin ratio technique (119). Environmental temperature and relative humidity were calculated using the National Weather Service monthly report (139, 140). Variances were analyzed (125) and means for the treatments were compared for significance by Duncan's multiple range test (61).
RESULTS AND DISCUSSION

Feed Consumption

Both the roughage and concentrate portions of all experimental diets were readily consumed at the beginning of the experiment. However, animals on 8% TF tended to consume the concentrate portion of their diet at a slower rate compared to the other groups. This may indicate that the 8% TF diet was somewhat less palatable than the others. By the fourth week of the experiment, daily environmental temperature and relative humidity began to increase dramatically (Table 3) and resulted in reduced roughage consumption on all experimental rations. Roughage refusal was highest on 8% F and lowest on the control group. However, cows on the 4% T refused more hay than cows on either 4% TF or 8% TF.

The reduction in roughage intake by the fourth week of the experiment led us to reduce daily hay allowance by 20% and the rations then became 56% concentrate and 44% roughage. Following this reduction the cows consumed all of their rations except for the 8% T group which refused 6% of the roughage diet the last two weeks of the experiment. Palmquist and Oelberg (150) reported that calcium soaps of tallow, fat blend or soya oil fed as 12% of the concentrate diet to high producing lactating cows had no effect on the pattern of feed intake. However, high environmental temperature and relative humidity have been shown to have an inhibitory effect on feed consumption (122, 163, 170).
Table 3. Average Daily Environmental Temperature (C°) and Relative Humidity (%) During the Experimental Period.

<table>
<thead>
<tr>
<th>Day</th>
<th>Average Daily Temperature (C°)</th>
<th>Average Daily Relative Humidity (%)</th>
<th>Day</th>
<th>Average Daily Temperature (C°)</th>
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</table>
Reduction in feed intake with increasing dietary fat has been shown by others (24, 59, 72, 76, 96, 153, 164, 171, 177, 191). Although the 8% TF diet contained 1.5 times as much fat as the 4% T diet (Table 2, CMH values), the 4% T group refused more roughage than the 8% TF group. This may suggest that hay refusal was associated with other factors besides environmental temperature, relative humidity and the energy content of the diet. Johnson and McClure (96) indicated that the depression in voluntary feed intake at high levels of dietary fat may be related to the formation of fecal soaps which reduce the availability of calcium and magnesium for the rumen microorganisms. Fecal soaps (Table 11) of the 8% T group were significantly (p<0.05) higher than the other groups, there being no significant differences (p<0.05) among the other groups. Changes in rumen fermentation may have contributed to the depression in feed consumption (24, 99, 164, 202). Bines et al (24) reported that feed intake from both roughage and concentrate were reduced with increasing dietary protected-soybean-tallow. Changes in rumen VFA, reduction in the digestibility of fiber and reduction in the output of short-chain fatty acids in milk fat which they attributed to the incomplete protection of dietary fat led them to their conclusion. More recently, Chalupa et al (38) found that the rumen acetate/propionate ratio was reduced 48% by feeding 10% tallow triglycerides. However, the ratio was reduced only 16% by feeding 10% tallow triglycerides or vegetable fats in the form of calcium salts. Both the acetate/propionate ratio in the rumen fluid (Table 6) and fiber digestibility (Table 5) were lowest on the 8% T diet of the herein reported experiment.
Body Weights of the Experimental Cows

The experimental cows showed no health problems related to the feeding of the experimental diets. Initial and final body weights of the cows are given in Table 4. There were no significant (p<0.05) differences among the five groups of cows in initial or final body weights. Changes in body weights over time on the individual experimental diets were also not significant (p>0.05).

Nutrient Digestibilities

Mean nutrient digestibilities of the experimental rations are shown in Table 5. There were significant differences (p<0.05) observed in the digestibility of ADF, cellulose, EE and chloroform-methanol lipid extract. However, when comparing the four dietary fat treatments, a general trend to reduce digestibility of nutrients with increasing dietary fat and with the inclusion of the free form of fat was noted. The only exception was in the digestibility of EE which will be discussed later.

Dry Matter Digestibility (DM)

Although not significant (p>0.05) DM digestibility of 4% TF increased by about 6% over the control group. Dry matter digestibility of 8% F however, fell below that of the control group. Depression in DM digestibility by dietary fat has been previously observed (24, 31, 33, 53, 54, 65, 67, 90, 96, 203, 215). This was especially true with high levels of the free form of fat (33, 65, 67) and with high degrees of unsaturation (33, 52, 53, 90, 96, 203, 215). On the other hand, low levels of dietary fat or protected forms of fat had either no effect
Table 4. Average Initial and Final Body Weights of the Experimental Cows (lbs).

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Initial Body Weight</th>
<th>Final Body Weight</th>
<th>Cow No.</th>
<th>Initial Body Weight</th>
<th>Final Body Weight</th>
<th>Cow No.</th>
<th>Initial Body Weight</th>
<th>Final Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>713</td>
<td>1345</td>
<td>1285</td>
<td>698</td>
<td>1463</td>
<td>1415</td>
<td>702</td>
<td>1465</td>
<td>1505</td>
</tr>
<tr>
<td>762</td>
<td>1348</td>
<td>1357</td>
<td>833</td>
<td>1370</td>
<td>1413</td>
<td>748</td>
<td>1353</td>
<td>1358</td>
</tr>
<tr>
<td>874</td>
<td>1233</td>
<td>1265</td>
<td>867</td>
<td>1260</td>
<td>1293</td>
<td>771</td>
<td>1478</td>
<td>1435</td>
</tr>
<tr>
<td>1058</td>
<td>1388</td>
<td>1372</td>
<td>1035</td>
<td>1530</td>
<td>1533</td>
<td>870</td>
<td>1223</td>
<td>1232</td>
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<td>1063</td>
<td>1520</td>
<td>1483</td>
<td>1038</td>
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<td>1493</td>
<td>1032</td>
<td>1618</td>
<td>1635</td>
</tr>
<tr>
<td>AVG.</td>
<td>1366</td>
<td>1352</td>
<td>1428</td>
<td>1429</td>
<td>1427</td>
<td>1433</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Initial Body Weight</th>
<th>Final Body Weight</th>
<th>Cow No.</th>
<th>Initial Body Weight</th>
<th>Final Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% F</td>
<td></td>
<td></td>
<td>8% F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>636</td>
<td>1558</td>
<td>1500</td>
<td>754</td>
<td>1455</td>
<td>1425</td>
</tr>
<tr>
<td>784</td>
<td>1490</td>
<td>1503</td>
<td>798</td>
<td>1410</td>
<td>1453</td>
</tr>
<tr>
<td>802</td>
<td>1395</td>
<td>1397</td>
<td>892</td>
<td>1328</td>
<td>1397</td>
</tr>
<tr>
<td>880</td>
<td>1400</td>
<td>1393</td>
<td>1008</td>
<td>1461</td>
<td>1418</td>
</tr>
<tr>
<td>1045</td>
<td>1438</td>
<td>1423</td>
<td>1050</td>
<td>1350</td>
<td>1338</td>
</tr>
<tr>
<td>AVG.</td>
<td>1456</td>
<td>1443</td>
<td></td>
<td>1401</td>
<td>1406</td>
</tr>
</tbody>
</table>
Table 5. Mean Digestibilities of Experimental Rations.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percent Digestibility</th>
<th>F Value</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>4% TF</td>
<td>8% TF</td>
</tr>
<tr>
<td>Dry matter</td>
<td>61.73</td>
<td>65.24</td>
<td>61.79</td>
</tr>
<tr>
<td>NDF</td>
<td>28.51</td>
<td>33.70</td>
<td>31.47</td>
</tr>
<tr>
<td>ADF</td>
<td>20.99&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>28.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.37&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cell-solubles</td>
<td>79.89</td>
<td>81.47</td>
<td>77.58</td>
</tr>
<tr>
<td>Cellulose</td>
<td>32.15&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>40.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>41.71</td>
<td>42.59</td>
<td>40.33</td>
</tr>
<tr>
<td>Ash</td>
<td>39.88</td>
<td>46.38</td>
<td>43.02</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>70.62</td>
<td>72.78</td>
<td>68.39</td>
</tr>
<tr>
<td>EE</td>
<td>71.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CM</td>
<td>60.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMH</td>
<td>54.14</td>
<td>65.01</td>
<td>62.83</td>
</tr>
<tr>
<td>Combustible energy</td>
<td>62.75</td>
<td>66.54</td>
<td>64.15</td>
</tr>
</tbody>
</table>

Values within a line with different superscripts are significantly different (p<0.05).
on DM digestibility (39, 85, 96, 126, 171) or may have actually improved it (100, 203).

Fiber Digestibilities

Acid-detergent fiber (ADF) digestibility was significantly (p<0.05) improved by the addition of 4% TF to the concentrate diet when compared to the control diet. However, ADF digestibility with the 8% F diet while not being significantly different (p>0.05) than the control diet was significantly (p<0.05) lower than the other three diets. No other differences in ADF digestibilities between treatments were significant.

All changes in ADF digestibility were in the cellulose portion rather than the hemicellulose portion of the cell wall components. Digestibility of cellulose of both the 4% TF and 8% TF diets was significantly (p<0.05) higher than that of both the control and the 8% F diets, but was not significantly different (p<0.05) from the 4% F diet. This indicates that the inclusion of calcium treated fat in the diet improved cellulose digestibility, possibly by preventing the detrimental effect of fat on the microorganisms that are concerned with cellulose digestion in the rumen.

Depression of fiber digestion with the inclusion of free forms of fats and oils in the diet has been previously confirmed (34, 56, 65, 67, 90, 96, 108, 110, 120, 144, 159, 220). Inclusion of protected-fats in the diet however did not effect fiber digestibility (59, 113). Jenkins and Palmquist (93) showed that substitution of the concentrate mixture with 10% tallow fatty acids resulted in a reduction in the digestibility of ADF. However, substitution with 10% tallow as verxite
(a non-nutritive carrier) or as calcium salts of fatty acids resulted in no change in ADF digestibility. Recently, calcium soaps of tallow fatty acids produced normal fiber digestibility of diets containing alfalfa pellets, concentrate and silage and fed to lactating cows (94).

Mineral Digestibility

Apparent mineral (ash) digestibility was non-significantly (p>0.05) reduced by the addition of 8% F to the diet, whereas with 4% TF and 8% TF it was non-significantly (p>0.05) improved compared to the control diet. Ash digestibility of 4% F was similar to that of the control diet. The reduction in ash digestibility with the 8% F diet was possibly due to high fecal soap excretion associated with the high level of dietary fat. Dietary fat addition has tended to reduce mineral digestion (24, 54, 100, 157, 171). The reduction was related to increased excretion of fecal soaps (100). The diet containing 8% F had a significantly (p<0.05) higher level of fecal soap compared to the others. Jenkins and Palmquist (94) reported lower mineral digestibility from the addition of calcium soaps of tallow to the diet of dairy cows.

Protein Digestibility

Crude protein (CP) digestibility was not affected (p>0.05) by dietary treatment, but the 8% F diet did tend to reduce it. Perry and Stewart (157) found that 3% animal fat reduced CP digestibility. Others (126, 146) reported no effect of tallow or blended-animal and vegetable fat on CP digestibility of the diet.
Lipid Digestibility

Apparent digestibility of lipid was calculated using either EE, CM or CMH values. Digestibility of EE was significantly (p<0.05) increased by the addition of either the calcium treated or untreated animal fat when compared to the control diet. Also EE digestibility was increased with increasing levels of dietary fat. This is in complete agreement with most of the literature (54, 67, 69, 108, 117, 126, 145, 159, 171). Using CM values, digestibility of lipid was also significantly (p<0.05) increased by the addition of both forms of animal fat, however the values were lower than their corresponding values for EE. On the other hand, CMH digestibility was not significantly different (p>0.05) among any of the treatments and their values were lower than those of CM and EE.

The differences among the three types of lipid digestibility are related to fecal soap excretion which can be extracted by CMH and to a lesser extent by CM but are not extracted by EE.

Although not significant (p>0.05), lipid digestibility (CMH) in the 8% F diet was lowest when compared to 8% TF, 4% TF and 4% F diets indicating high levels of fecal soap excretion. In addition, CMH digestibility was highest on 4% TF and 8% TF indicating less fecal soap excretion and higher absorption of lipid with calcium treated fat feeding. Figroid (72) found that CMH digestibility was reduced with increased animal fat feeding to beef steers. He also reported that the higher the dietary fat the higher the fecal soap excretion.
Gross Energy Digestibility

Even though there were no significant differences (p>0.05) among treatments in gross energy (GE) digestibility the highest value was from the 4% TF diet, the lowest from the 8% F diet. The high GE digestibility from 4% TF further indicates a higher lipid utilization and less energy loss from fecal soaps compared to the 8% F diet. Reduction in total energy digestibility with increasing dietary animal fat (72) or protected soybean-tallow (24) has been shown. Other studies (59, 69, 145, 146, 171) reported no significant effect of dietary fat on energy digestibility.

Volatile Fatty Acids of the Rumen Fluid

Molar percentages of rumen VFA are given in Table 6. The molar percentage of rumen acetate was significantly (p<0.05) reduced on the 8% F diet compared to the 4% TF and 4% F diets. All other differences among treatments were not significant (p>0.05). On the other hand, the molar proportion of rumen propionate was significantly (p<0.05) higher on the 8% F diet compared to the 4% TF, 8% TF and 4% F diets but was not significantly (p>0.05) different than the control diet. There were no significant (p>0.05) differences among treatments in any of the other rumen VFA, however, rumen butyrate tended to be higher on the 4% TF, 8% TF and 4% F diets compared to the control and 8% F diets. The rumen acetate/propionate ratio was significantly (p<0.05) lower on the 8% F diet compared to the 4% TF, 8% TF and 4% F diets but it was not significantly (p<0.05) different than the control diet.

The reduction in rumen acetate/propionate ratio on the 8% F diet could be related to the lower roughage intake of this group.
Table 6. Mean Molar Percentages of Volatile Fatty Acids (VFA) of Rumen Fluid from Cows Fed Experimental Rations.

<table>
<thead>
<tr>
<th>VFA</th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
<th>F Value</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>37.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.99&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.97</td>
<td>1.37</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>30.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>23.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.37</td>
<td>2.52</td>
</tr>
<tr>
<td>Iso-butyric acid</td>
<td>0.85</td>
<td>1.40</td>
<td>1.18</td>
<td>1.33</td>
<td>1.05</td>
<td>1.99</td>
<td>0.16</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>22.31</td>
<td>26.67</td>
<td>25.19</td>
<td>24.77</td>
<td>20.79</td>
<td>1.91</td>
<td>1.71</td>
</tr>
<tr>
<td>Iso-valeric acid</td>
<td>2.87</td>
<td>2.96</td>
<td>2.79</td>
<td>3.18</td>
<td>2.31</td>
<td>0.76</td>
<td>0.37</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>5.49</td>
<td>4.21</td>
<td>4.38</td>
<td>4.20</td>
<td>4.94</td>
<td>1.26</td>
<td>0.50</td>
</tr>
<tr>
<td>Acetic acid/Propionic acid ratio</td>
<td>1.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.08</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values within a line with different superscripts are significantly different (p<0.05).
Cows on the 8% F diet refused 6% of the roughage during the last two weeks of the experiment. High-grain, restricted-roughage diets have been shown to reduce rumen acetate/propionate ratios (17, 18, 35, 181, 202). Since cows on the 4% TF, 8% TF and 4% F diets consumed their roughage normally during the last two weeks of the experiment and as the rumen acetate/propionate ratio of 8% TF was lower than both the 4% TF and 4% F diets, the reduction in rumen acetate/propionate ratio could also be related to the high dietary fat addition. Narrow acetate/propionate ratios with the inclusion of dietary fat has been shown in the literature (17, 36, 37, 38, 90, 99, 137, 172, 185, 191, 193, 204). Acetate/propionate ratios from the rumen fluid of the 4% TF diet was higher than the 8% TF diet. This would seem to indicate that calcium treated animal fat was not completely protected in the rumen at the higher level of supplementation. It may have partially hydrolyzed with the resulting free fatty acids adversely affecting rumen environment resulting in a lower rumen acetate/propionate ratio. This is in agreement with the results of Chalupa et al (38) who found that 10% tallow reduced the acetate/propionate ratio by 48%, whereas 10% calcium salts of tallow reduced it by only 16%.

**Serum-Total Lipid and Serum-Total Cholesterol**

Mean serum-total lipid and serum-total cholesterol for the pre-trial, third and sixth week of the experiment are shown in Table 7 and Figures 1 and 2. The experimental cows were consuming high amounts (about 8 pounds/cow/day) of whole cottonseed prior to the beginning of the trial which apparently caused their serum-total lipids and total-cholesterol to be high for the pretrial collection.
Table 7. Mean Serum-Total Lipids and Serum Total Cholesterol From Cows Fed the Experimental Rations (mg/100 ml).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Total Lipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretrial</td>
<td>776</td>
<td>866</td>
<td>776</td>
<td>754</td>
<td>864</td>
</tr>
<tr>
<td>Third Week</td>
<td>546&lt;sup&gt;b&lt;/sup&gt;</td>
<td>742&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>770&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>634&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>830&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sixth Week</td>
<td>424&lt;sup&gt;b&lt;/sup&gt;</td>
<td>730&lt;sup&gt;a&lt;/sup&gt;</td>
<td>688&lt;sup&gt;a&lt;/sup&gt;</td>
<td>580&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>616&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum Total Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretrial</td>
<td>294</td>
<td>324</td>
<td>316&lt;sup&gt;·&lt;/sup&gt;</td>
<td>265</td>
<td>344</td>
</tr>
<tr>
<td>Third Week</td>
<td>327</td>
<td>296</td>
<td>319</td>
<td>267</td>
<td>337</td>
</tr>
<tr>
<td>Sixth Week</td>
<td>159&lt;sup&gt;b&lt;/sup&gt;</td>
<td>269&lt;sup&gt;a&lt;/sup&gt;</td>
<td>283&lt;sup&gt;a&lt;/sup&gt;</td>
<td>201&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>235&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values within a line with different superscripts are significantly different (p<0.05).
There were no significant (p>0.05) differences among the five treatments in serum-total lipids or serum-total cholesterol from the pretrial period. However, by the end of the experiment serum-total lipids and serum-total cholesterol of the 4% TF and 8% TF diets were significantly (p<0.05) higher than those of the control diet. There were no other significant (p>0.05) differences in serum-total lipids or serum-total cholesterol. There were, however, significant (p<0.05) interactions between treatments and time on treatments (week). Comparing the serum-total lipids and serum-total cholesterol for the pretrial and the final week, the control, 4% TF, 4% F and 8% F diets produced values that were significantly (p<0.05) reduced by time, whereas the 8% TF diet maintained higher values of serum-total lipids and serum-total cholesterol. It can be also seen from Figures 1 and 2 that animals on 4% and 8% calcium treated animal fat had higher values for serum-total lipids and serum-total cholesterol compared to the other animals by the end of the experiment. This indicates that animals on calcium treated animal fat were able to absorb more of the dietary animal fat compared to the animals on the untreated fat diets.

Increasing serum-total lipids of lactating cows with the addition of fat to the diet has been shown by others (1, 113, 145, 149, 162, 201, 224). Other studies (1, 26, 81, 84, 101, 112, 113, 162, 171, 177, 224, 225, 226, 227, 228) have shown that serum-cholesterol also tended to increase with the dietary addition of fats to the rations of lactating cows. The elevation in serum cholesterol is an obligatory response to facilitate the transport of the increasing plasma lipids (226). Nestel et al (136) indicated that increased intestinal
synthesis of cholesterol with fat feeding was a mechanism used to facilitate the absorption of long-chain fatty acids.

**Fatty Acids Composition of Serum-Total Lipids**

Mean percentages of fatty acids of serum total lipids for the pretrial, third and sixth week of the experiment are given in Table 8. Although there were no significant differences (p>0.05) among treatments in fatty acid composition of serum-total lipids in the pretrial period, the individual experimental cows showed marked differences in their fatty acid profiles (data are not shown for the individual cow but rather for the group averages). This was particularly true for the C16:0, C18:0 and C18:2 fatty acids. For instance, C18:2 was as high as 65% and as low as 12% of the total plasma fatty acids. This is in complete disagreement with the literature where the major plasma fatty acid of lactating cows regardless of dietary feeding was shown to be C18:2 and accounted for about 40-55% of total plasma fatty acids (112, 132, 188). The remarkable low levels of C18:2 fatty acids of the plasma in some of the experimental cows in the pretrial period cannot be explained. However, the decrease in C18:2 was associated with marked increases in C16:0 and C18:0.

Values for the third and sixth week of the experiment for the total plasma fatty acids were more consistent within the same group of cows. However, on the last week of the experiment one cow from the control group gave a completely different set of values as compared to the other cows of her group. Where the other four cows had about 65% of their plasma fatty acids as C18:2, she had only about 20%.
Figure 1. Mean Serum-Total Lipids from Cows Fed the Experimental Rations.
Figure 2. Mean Serum-Total Cholesterol from Cows Fed the Experimental Rations.
Table 8. Mean Percentages of Fatty Acids of Serum Total Lipids From Cows Fed the Experimental Rations (Percent of Total).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pretrial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>18.06</td>
<td>23.41</td>
<td>17.08</td>
<td>22.77</td>
<td>19.61</td>
</tr>
<tr>
<td>C16:1</td>
<td>4.05</td>
<td>2.77</td>
<td>1.56</td>
<td>0.44</td>
<td>3.57</td>
</tr>
<tr>
<td>C18:0</td>
<td>26.79</td>
<td>36.32</td>
<td>24.67</td>
<td>29.09</td>
<td>30.64</td>
</tr>
<tr>
<td>C18:1</td>
<td>8.94</td>
<td>10.18</td>
<td>8.23</td>
<td>10.19</td>
<td>9.42</td>
</tr>
<tr>
<td>C18:2</td>
<td>40.24</td>
<td>26.30</td>
<td>45.81</td>
<td>35.28</td>
<td>35.12</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.93</td>
<td>1.02</td>
<td>2.65</td>
<td>2.23</td>
<td>1.65</td>
</tr>
<tr>
<td><strong>3rd Week</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>11.19</td>
<td>9.77</td>
<td>10.45</td>
<td>11.23</td>
<td>10.13</td>
</tr>
<tr>
<td>C16:1</td>
<td>4.36</td>
<td>2.72</td>
<td>3.17</td>
<td>2.65</td>
<td>3.55</td>
</tr>
<tr>
<td>C18:1</td>
<td>4.82b</td>
<td>6.15ab</td>
<td>9.08a</td>
<td>6.62ab</td>
<td>9.73a</td>
</tr>
<tr>
<td>C18:2</td>
<td>57.00</td>
<td>61.30</td>
<td>56.71</td>
<td>59.49</td>
<td>55.92</td>
</tr>
<tr>
<td>C18:3</td>
<td>5.62</td>
<td>6.20</td>
<td>8.09</td>
<td>5.90</td>
<td>7.00</td>
</tr>
<tr>
<td><strong>6th Week</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td>13.63</td>
<td>10.51</td>
<td>11.08</td>
<td>11.25</td>
<td>11.02</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.93ab</td>
<td>0.85b</td>
<td>1.95ab</td>
<td>2.09ab</td>
<td>3.16a</td>
</tr>
<tr>
<td>C18:0</td>
<td>19.02</td>
<td>14.39</td>
<td>12.87</td>
<td>14.04</td>
<td>12.90</td>
</tr>
<tr>
<td>C18:1</td>
<td>6.02b</td>
<td>7.38b</td>
<td>9.80ab</td>
<td>7.84b</td>
<td>12.82a</td>
</tr>
<tr>
<td>C18:2</td>
<td>56.04</td>
<td>61.97</td>
<td>60.16</td>
<td>60.52</td>
<td>55.61</td>
</tr>
<tr>
<td>C18:3</td>
<td>3.37</td>
<td>4.90</td>
<td>4.14</td>
<td>4.29</td>
<td>4.50</td>
</tr>
</tbody>
</table>

Values within a line with different superscripts are significantly different (p<0.05).
The only major significant difference (p<0.05) in plasma fatty acids related to dietary fat feeding was in C18:1 which increased with increasing intake of both animal fat and calcium treated animal fat. This was expected since about 47% of the fatty acids of the dietary fat was C18:1 (Table 13). Increased plasma fatty acids associated with increased dietary feeding was observed in lactating cows by Storry et al (199). Plasma fatty acids from the third and sixth week of the experiment showed that regardless of dietary treatment, the major plasma fatty acid was C18:2 which accounted for about 55-62% of the total. The values of C18:2 from the third and sixth week of the experiment were higher than their correspondent values from the pretrial. The increase in C18:2 was associated with decreases in the concentrations of C16:0 and C18:0.

**Milk Yield and Milk Composition**

Mean yields and composition of milk are given in Table 9 and Figures 3, 4, 5 and 6.

**Milk Yield**

There were no significant (p>0.05) differences among treatments in daily milk production. Diets supplemented with untreated or calcium treated animal fat did produce about 9 and 3.5% respectively more milk than the control diet. The weekly effect of diet on the amount of milk produced is shown in Figure 3. All experimental cows produced milk normally through the third week of the experiment where increasing environmental temperatures and relative humidity (Table 3) dramatically increased and caused a pronounced decrease in daily milk
Table 9. Mean Yield and Composition of Milk of Cows Fed the Experimental Rations.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
<th>F Value</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily milk yield (lbs)</td>
<td>65.00</td>
<td>67.27</td>
<td>70.88</td>
<td>71.01</td>
<td>69.47</td>
<td>0.24</td>
<td>5.24</td>
</tr>
<tr>
<td>Milk fat (%)</td>
<td>2.63</td>
<td>2.82</td>
<td>2.87</td>
<td>3.07</td>
<td>2.94</td>
<td>0.82</td>
<td>0.18</td>
</tr>
<tr>
<td>Solid-not-fat (%)</td>
<td>8.22</td>
<td>7.91</td>
<td>7.96</td>
<td>8.12</td>
<td>8.13</td>
<td>1.08</td>
<td>1.24</td>
</tr>
<tr>
<td>Milk protein (%)</td>
<td>2.73</td>
<td>2.62</td>
<td>2.66</td>
<td>2.60</td>
<td>2.61</td>
<td>1.26</td>
<td>0.48</td>
</tr>
</tbody>
</table>
production. The reduction in daily milk yield was associated with a reduction in roughage intake. The depression in milk yield was highest for the 8% F group and seems to have been associated with low roughage intake, reduced digestibility of the ration components especially crude fiber and possibly high fecal soap excretion.

Reduction of milk yield with increasing environmental temperature has been observed by others (32, 106, 122, 179, 201). This could be due to an indirect effect by a reduction in feed consumption and/or a direct one through the cow's physiological responses in her attempt to maintain heat balance (32, 170, 212).

Dietary tallow feeding has been shown by some researchers to have no significant effect on milk yield in both the free and protected forms (35, 64, 88, 111, 113, 126, 134, 171, 177, 184, 222). However, others have reported increases in milk yield by the feeding of free tallow (1, 14, 201, 224) or protected tallow (134, 224). Palmquist and Conrad (193) reported that 10% tallow lowered milk yield and was related to lower feed intake and an increase in body weight. In our study, there were no significant (p>0.05) changes in body weight of the experimental cows. However there was a reduction in forage consumption with the 8% F group.

Milk Fat Content

Average weekly milk fat percentages from the experimental diets are shown in Figure 4. The general increase in milk fat content between the third and fifth week of the experiment was undoubtedly due to the severe depression in milk yield during this period.
Figure 3. Average Daily Milk Production by Week from Cows Fed the Experimental Rations.
No significant (p>0.05) differences in average daily milk fat percentages were observed. However, milk fat content of the 4% and 8% TF diets were 7% and 9% higher than the control diet respectively. The 4% and 8% F diets produced 17% and 11% respectively more milk fat content than the control diet. The relatively low levels of milk fat percentages on all experimental rations may have been due to relatively high average daily milk yield (30-32 kg/cow/day).

The non-significant change in milk fat content was in agreement with many other studies involving the use of various levels of dietary tallow (14, 88, 111, 134, 184, 197, 201). However, Brown et al (35) reported significant increases in milk fat content by inclusion of 6% tallow in the concentrate portion of a relatively high-roughage diet. Significant improvement in milk fat content was also observed by feeding formaldehyde-treated tallow (64, 113, 171). Conversely, Adams et al (1) reported a reduction in milk fat content by feeding cows 10% tallow.

Milk Fatty Acids

Mean percentages of milk fatty acids for the pretrial, third and sixth weeks of the experimental period are given in Table 10. Because of the analytical technique used butyric (C4:0) and caproic acids (C6:0) were not detected in most of the samples examined. No significant (p<0.05) differences were found in fatty acid percentage among the experimental diets from the pretrial period. Milk samples from the third week showed a significant (p<0.05) increase in C18:1 with the feeding of both untreated and calcium treated animal fat in comparison to the control samples. There were also significant (p<0.05) reductions in fatty acids between C10 and C16:0 with the feeding of
Figure 4. Average Milk Fat Percent from Cows Fed the Experimental Rations.
Figure 5. Average Milk Solids-Not-Fat (SNF) Percent from Cows Fed the Experimental Rations.
Figure 6. Average Milk Protein Percent from Cows Fed the Experimental Rations.
Table 10. Mean Percentages of Milk Fatty Acids from Cows Fed the Experimental Rations (Percent of Total).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pretrial</td>
<td></td>
<td>3rd Week</td>
<td>6th Week</td>
</tr>
<tr>
<td>C6:0</td>
<td>0.15</td>
<td>0.16</td>
<td>0.21</td>
<td>0.60</td>
<td>0.45</td>
</tr>
<tr>
<td>C8:0</td>
<td>1.02</td>
<td>0.98</td>
<td>0.59</td>
<td>0.70</td>
<td>0.55</td>
</tr>
<tr>
<td>C10:0</td>
<td>2.14</td>
<td>3.11</td>
<td>2.21</td>
<td>2.95</td>
<td>3.32</td>
</tr>
<tr>
<td>C12:0</td>
<td>3.29</td>
<td>3.67</td>
<td>2.68</td>
<td>3.11</td>
<td>3.83</td>
</tr>
<tr>
<td>C14:0</td>
<td>10.95</td>
<td>11.66</td>
<td>9.93</td>
<td>10.95</td>
<td>11.66</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.98</td>
<td>1.13</td>
<td>0.97</td>
<td>0.73</td>
<td>0.99</td>
</tr>
<tr>
<td>C16:0</td>
<td>27.17</td>
<td>27.73</td>
<td>27.28</td>
<td>26.53</td>
<td>27.38</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.83</td>
<td>1.99</td>
<td>2.36</td>
<td>1.99</td>
<td>1.99</td>
</tr>
<tr>
<td>C18:1</td>
<td>28.23</td>
<td>26.38</td>
<td>27.35</td>
<td>27.35</td>
<td>27.35</td>
</tr>
<tr>
<td>C18:2</td>
<td>4.91</td>
<td>4.79</td>
<td>4.45</td>
<td>4.45</td>
<td>4.45</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.76</td>
<td>1.89</td>
<td>1.91</td>
<td>1.91</td>
<td>1.91</td>
</tr>
</tbody>
</table>

Values within a line with different superscripts are significantly different (p<0.05).
the same diets. There were no significant changes in the percentage of any other fatty acids, although dietary fat tended to increase C18:0 and C16:1 fatty acids compared to the control diet. Similar changes were observed in the percentages of milk fatty acids during the last week of the experiment.

The reductions in short and intermediate chain fatty acids with dietary fat were observed by others (13, 15, 35, 84, 112, 117, 154, 160, 162, 171, 181, 184, 185, 186, 193, 194), however, in this experiment C6:0-C8:0 were not affected by dietary fat suggesting their partial or complete independence from the malonyl CoA pathway (15, 117, 196). Since the change in rumen VFA was not very dramatic, the reduction in the C10-C16:0 acids then must be related to increased uptake of long-chain fatty acids by the mammary gland. This would in turn cause inhibition of acetyl CoA carboxylase (86, 89, 178) which catalyzes the rate limiting step in fatty acid synthesis in the bovine mammary gland (74). This mechanism was previously suggested by Moore and Steele (128).

The increase in C18:1 acid of milk fat with both forms of animal fat feeding could be through increased uptake of this acid from plasma triglycerides, or through desaturation of C18:0 in the epithelial cells of the small intestine (20, 22) or in the mammary gland (19, 21, 41, 105). Although the main circulating fatty acid of the plasma was C18:2 on all experimental diets, this acid was not significantly (p<0.05) increased in milk fat. This was as expected because C18:2 is mainly associated with plasma phospholipids and cholesterol esters (112, 132, 188) which do not contribute their fatty acids to the mammary gland (6).
Milk Protein and Solids-Not-Fat

Average weekly percentages of milk protein and SNF are given in Figures 5 and 6. Milk protein values for the pretrial period were not determined for technical reasons.

There were no significant (p>0.05) differences among dietary treatments in protein or SNF content of the milk. Milk protein did tend to decrease with both forms of dietary animal fat. Reduction in milk protein has been previously observed by others with both normal fat (14, 111, 176, 180) and protected fat (64, 113, 117, 134, 149, 171, 191) feeding.

Fecal Soaps Excretion

Fecal soaps as a percent of fecal dry matter are shown in Table 11. Fecal soaps from the 8%F diet were significantly higher (p<0.05) compared to those of the other experimental diets. There were no significant (p>0.05) differences between the other diets. However the 4% TF group of cows tended to excrete less fecal soaps than the control group. Animals on 8% F excreted 35% more fecal soaps compared to animals on 8% TF. Increasing fecal soap excretion with increasing dietary fat is in agreement with others (69, 72, 96).

Fatty Acids of the Feces

Mean percentages of fecal fatty acids are given in Table 12. Stearic acid excretion was significantly (p<0.05) increased with the feeding of both forms of animal fat. The increase in C18:0 excretion was associated with decreased excretion of C18:1 and C18:2 fatty acids. Excretion of C18:1 fatty acid was the lowest on the calcium treated
animal fat diets compared to their correspondent untreated fat diets suggesting increased absorption of fatty acids with calcium treated animal fat feeding.
Table 11. Ether Extracts (EE), Chloroform-Methanol (CM), Chloroform-Methanol-Hydrochloric Acid (CMH) and Fecal Soaps* as a Percent of Fecal Dry Matter from Cows Red the Experimental Rations.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE</td>
<td>2.00</td>
<td>1.83</td>
<td>1.94</td>
<td>2.01</td>
<td>2.09</td>
</tr>
<tr>
<td>CM</td>
<td>4.26</td>
<td>4.92</td>
<td>5.59</td>
<td>5.36</td>
<td>5.79</td>
</tr>
<tr>
<td>CMH</td>
<td>9.37</td>
<td>9.87</td>
<td>11.50</td>
<td>10.73</td>
<td>13.76</td>
</tr>
<tr>
<td>Fecal Soaps</td>
<td>5.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.37</td>
<td>7.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*CMH minus CM

a, b - Values within a line with different superscripts are significantly different (p<0.05).
Table 12. Mean Percentages of Fatty Acids of Feces From Cows Fed the Experimental Rations.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
<th>F Value</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.62</td>
<td>0.51</td>
<td>0.94</td>
<td>0.55</td>
<td>0.32</td>
<td>0.54</td>
<td>0.31</td>
</tr>
<tr>
<td>C14:1</td>
<td>1.07</td>
<td>0.57</td>
<td>0.61</td>
<td>0.27</td>
<td>0.09</td>
<td>1.04</td>
<td>0.37</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.20</td>
<td>24.86</td>
<td>25.52</td>
<td>23.39</td>
<td>25.31</td>
<td>0.67</td>
<td>1.06</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.31</td>
<td>0.59</td>
<td>1.20</td>
<td>0.61</td>
<td>0.76</td>
<td>1.12</td>
<td>0.31</td>
</tr>
<tr>
<td>C18:0</td>
<td>34.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.39</td>
<td>3.36</td>
</tr>
<tr>
<td>C18:1</td>
<td>14.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.88&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>9.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.43</td>
<td>0.94</td>
</tr>
<tr>
<td>C18:2</td>
<td>18.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.08&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>8.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.89</td>
<td>1.02</td>
</tr>
<tr>
<td>C18:3</td>
<td>6.11</td>
<td>2.52</td>
<td>2.64</td>
<td>2.48</td>
<td>0.46</td>
<td>2.49</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Values within a line with different superscripts are significantly different (p<0.05).
Table 13. Mean Percentages of Fatty Acids of Alfalfa Hay, Animal Fat and Experimental Concentrates.

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Animal Fat</th>
<th>Alfalfa Hay</th>
<th>Control</th>
<th>4% TF</th>
<th>8% TF</th>
<th>4% F</th>
<th>8% F</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>3.07</td>
<td>0.99</td>
<td>0.21</td>
<td>1.99</td>
<td>2.58</td>
<td>2.09</td>
<td>2.61</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.62</td>
<td>-</td>
<td>-</td>
<td>0.36</td>
<td>0.55</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>C16:0</td>
<td>27.57</td>
<td>30.19</td>
<td>18.96</td>
<td>22.48</td>
<td>23.99</td>
<td>22.56</td>
<td>23.10</td>
</tr>
<tr>
<td>C16:1</td>
<td>2.30</td>
<td>1.75</td>
<td>-</td>
<td>2.28</td>
<td>2.87</td>
<td>2.28</td>
<td>2.86</td>
</tr>
<tr>
<td>C18:0</td>
<td>15.33</td>
<td>5.24</td>
<td>3.14</td>
<td>11.62</td>
<td>13.27</td>
<td>10.51</td>
<td>11.73</td>
</tr>
<tr>
<td>C18:1</td>
<td>46.87</td>
<td>4.49</td>
<td>22.28</td>
<td>34.48</td>
<td>37.18</td>
<td>34.64</td>
<td>39.08</td>
</tr>
<tr>
<td>C18:2</td>
<td>3.14</td>
<td>19.76</td>
<td>51.27</td>
<td>22.52</td>
<td>15.53</td>
<td>23.59</td>
<td>14.70</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.64</td>
<td>36.29</td>
<td>4.05</td>
<td>2.35</td>
<td>1.86</td>
<td>2.34</td>
<td>3.70</td>
</tr>
</tbody>
</table>


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ruminants. V. Entry rate into the body and incorporation into

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