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The Lipid Composition of Milk as Affected by High Grain-Limited Roughage Rations Containing Whey Products

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THE LIPID COMPOSITION OF MILK AS AFFECTED BY HIGH GRAIN-LIMITED ROUGHAGE RATIONS CONTAINING WHEY PRODUCTS

BY

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HIGH GRAIN-LIMITED ROUGHAGE RATIONS
CONTAINING WHEY PRODUCTS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Dairy Science Department Date

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INTRODUCTION

Certain rations fed to lactating dairy cows appear to have a dramatic influence on the lipid composition of milk fat produced. A high grain ration fed with a limited amount of roughage tends to cause a depression in milk fat production while at the same time changing the milk fat composition.

There have been various attempts to alleviate the depression in milk fat. Minerals used in the ration seem to slightly relieve the milk fat depression caused by high grain rations. One drawback has been lower milk production due to a decline in concentrate consumption. Whey products added to a high concentrate ration produced more acceptable results. The milk fat production was returned nearly to normal without altering total milk production.

Previous research has been involved with the gross composition of milk and the fatty acid composition of the milk fat. Changes have been shown in the fatty acid composition of milk fat when cows were fed high concentrate rations. These changes may be responsible for flavor problems, such as a decreased oxidative stability of the milk. In light of this, the lipid composition of milk is of economic importance to the dairy industry.

The purpose of this research was to determine changes that occur in the lipid composition of milk obtained from cows on high grain-limited roughage rations containing whey products. The specific objectives of the study were:

1. To compare the phospholipid content of the milk fat in the standardization period to that during the experimental period.
2. To determine the total fatty acid composition of the milk fat in the standardization and experimental periods.
3. To determine the fatty acid composition of the polar lipid fraction.
4. To measure gross changes in the polar lipid components.

REVIEW OF LITERATURE

The effect of variations in the diet of lactating cows on the composition of milk has been studied since the early 1930's. Early work evaluated the effect of various types and amounts of vegetable oils and animal fats in the rations on the composition of milk fat and the properties of butter. Investigators (19, 27, 61) found that feeding soybean oil, linseed oil, tallow, or corn oil had an influence on the physical properties and chemical constants of the butter and butterfat. Results showed changes in the hardness of the butter. Linseed oil and corn oil decreased the hardness while the remaining fats and oils increased the hardness of the butter. It appeared that an increase in the quantity of unsaturated glycerides may have played an important role in the changes that occurred in the hardness of the butter.

Animal tallow added to the ration has been studied (1, 2, 54, 56, 58) to determine the effect on milk production and milk composition. The more saturated fats tend to increase milk fat production. Brown et al. (8) found that feeding tallow, although significantly increasing milk fat percentage, resulted in a decrease of the fatty acids caproic acid through myristic acid, and a general increase in stearic, oleic, and linoleic acids (C18 acids). It was noted that only lauric acid had a significant decrease caused by feeding tallow.

Unsaturated oils have the opposite effect by causing a depression in milk fat production (8, 31, 58). When feeding cottonseed oil in a low roughage diet, Brown et al. (8) found there was no change in

palmitic or palmitoleic acids (C16 acids) of the milk fat. The fatty acids from caproic acid through myristic acid decreased and the C18 acids increased. They concluded that the oil need not be present in high amounts to affect the milk fat. Williams et al. (61) indicated an increased milk production but decreased butterfat production and fat percentage while feeding soybean oil. They concluded that the method of feeding fat affects the nature of fat metabolism since the total unsaturation in the butterfat was greater when the soybean oil was fed than when cracked soybeans were fed.

Cod liver oil fed to dairy cows has also been shown to markedly reduce the fat percentage of milk (15, 35, 43, 48, 60). Hilditch (18), postulated that the depressing effect of cod liver oil is due to the unsaturated fatty acids of the arachidonic and behenic series exerting a poisoning effect on mammary enzyme systems. Beitz and Davis (5) in 1964 compiled data which also indicated the depressing effect of cod liver oil. They concluded that this depression in milk fat synthesis occurs somewhere beyond the rumen. They also found a significant decrease in stearic acid, and a significant increase in palmitoleic and linolenic acids in the milk fat, with linolenic acid increasing 200 per cent.

Contrary to earlier reports, the effect of oils on milk fat depression appear to have no influence on the enzyme system. Baldwin et al. (4) in 1969 stated there were no significant enzymatic changes which occurred in mammary tissue when milk fat percentage was depressed. An alteration in mammary gland metabolism may occur. When

they compared the concentrate diet to a hay diet, the concentrate diet caused increases in oleic and linoleic acid with the remaining fatty acids being slightly decreased. Their observations suggested that a part of the severe milk fat depression is due to a decrease in the availability of long-chain fatty acids for milk synthesis.

Tove and Mochrie (56) compared the effect of dietary soybean concentrate and an intravenously infused cottonseed oil emulsion on the composition of depot fat and milk fat. The addition of soybeans to the diet altered the fatty acid composition of both the depot fat and the milk fat. The milk fat produced had increases in stearic and oleic acids with a corresponding decrease in myristic and palmitic acids. The percentage of linoleic acid slightly increased. The changes in the depot fat of stearic and linoleic acid paralleled those observed in the milk fat, but myristic, palmitic, palmitoleic, and oleic acids in the depot fat behaved quite differently from those of the milk fat. Palmitic and myristic acids in the depot fat remained constant throughout the experiment. There was a significant decrease in palmitoleic acid and a lesser decrease in oleic acid in the depot fat. In the cottonseed oil emulsion infusion experiment, only the milk fat was analyzed. Immediately after the infusion there was a decrease in milk production which returned to normal by 24 hours after the infusion. There was no significant effect of the infusion on the milk fat percentage. Linoleic acid increased by 11.3 per cent and 19.8 per cent when intravenously infused with 450 grams or 900 grams (respectively) of a cottonseed oil emulsion. The linoleic acid level returned to normal after one day

with the 450 gram infusion, but the increased level of linoleic acid was maintained for several days with a 900 gram infusion. There was no change in the oleic acid level while all the remaining fatty acids analyzed decreased slightly. They concluded that these observations are indicative of the efficiency and completeness of hydrogenation by the rumen microorganisms.

Considering the possible relationships that oils have on the fatty acid composition of milk fat, it is important to study the effect of feeding cows high grain-limited roughage diets. It is known (5, 11, 16, 25, 26) that feeding high levels of concentrate with a restricted amount of roughage causes a depression in the fat content of the milk in addition to a decrease in total milk fat yield. Opstvedt and Ronning (40) found that a reduced output of milk fat was due to a reduction in the amounts of all major fatty acids synthesized. The most pronounced decrease was in the long-chained saturated acids. Of the few acids that showed a significant increase, linoleic showed the greatest increase. King and Hemken (28) reported very similar results but did not analyze any fatty acids shorter than lauric acid. They also found a marked decrease in palmitic and stearic acids with a smaller decrease in myristic acid. Lauric acid showed an increase with linolenic acid showing a slight increase. Kunsman and Keeney (30) found decreases in long-chain saturated acids and compensatory increases in oleic and linoleic acids. Beitz and Davis (5) found no significant difference between the control ration and the high grain ration for the fatty acids from caproic acid through myristic acid. They found a

significant decrease in palmitic and stearic acids with a significant increase in palmitoleic, oleic, and linoleic acids of which linoleic had the greatest increase. Jorgenson et al. (26), using a similar ration as those above, found a decrease in the short-chain fatty acids which included butyric, caproic, caprylic, and capric acids. Except for palmitic and stearic acids, which decreased in content, the remaining fatty acids analyzed increased during the experimental period compared to a standardization period. Linoleic acid had the greatest increase and stearic acid had the greatest decrease on a percentage basis.

Van Soest (58) reviewed the theories proposed as explanations for the depression of milk fat from feeding high levels of concentrate. Three precursors in the blood are mainly responsible for fatty acids of the milk lipids. These precursors are the chylomicrons and low density lipoproteins which give rise to various long-chain fatty acids, acetate, and beta-hydroxybutyrate. Milk fat depressing conditions have reduced outputs possibly due to a shortage of each of the precursors. One widely claimed theory is a deficiency of rumen acetic acid for milk fat synthesis in the mammary gland. Investigators (8, 24, 26, 28, 45) have found lowered molar proportions of acetate in the rumen liquor from cows fed high grain-limited roughage rations. Tyznik and Allen (57) showed that feeding sodium acetate to cows producing low-fat milk on a high concentrate-low roughage diet increased the milk fat to the normal level within 24 hours. The acetate feeding was then discontinued and the fat test immediately decreased to the sub-normal low

roughage level. Again, the acetate was fed and the milk fat had a sustained increase. This indicates the necessity of rumen acetic acid for sustained milk fat production.

The second theory involves observed increases of propionate in the rumen liquor and a reduction in the blood concentration of beta-hydroxybutyric acid (8, 24-26, 28, 29, 49). The antiketogenic activity of propionate provoked a shortage of beta-hydroxybutyrate which is believed responsible for the reduced fat test (47, 53).

The last theory discussed involved fat mobilization due to control from the endocrine system. McClymont and Vallance (32) suggested that increased blood glucose levels depressed the release of free fatty acids from adipose tissue, thus reducing the plasma lipids available for milk fat synthesis. Reduced levels of blood lipids (26, 32, 40, 53) have been observed which supports this theory. There have been observations in support of all the theories, but conclusive evidence for any one theory has been lacking.

There are several measures which can be taken to alleviate the situation when depressions in milk fat occur. Jorgenson and Schultz (25) noted only slight decreases in milk fat depression when pelleted hay and corn were fed in conjunction with baled hay. Limiting the hay fed and supplementing pelleted forage, O'Dell et al. (39) found no correction in the depressed milk fat tests but did prevent a further decline in milk fat test. They also found that, although feeding pelleted forages four times daily, only a partial correction of a decreased milk fat test was achieved. Chalupa et al. (9) alleviated

the milk fat depression caused by feeding pellets as the sole forage with the addition of supplemental conventional forages. They also showed a higher proportion of unsaturated fatty acids in the milk fat. The short-chain fatty acids, caproic acid through lauric acid, were significantly decreased as were myristic, palmitic and stearic acids. All the unsaturated fatty acids analyzed, with the exception of myristoleic acid, were found in significantly higher amounts.

Another measure to control depression in milk fat from high grain rations has been the addition of minerals or whey products to the ration. Studies since 1961 (12-14, 34, 55) have shown that using sodium or potassium bicarbonate and magnesium oxide, partially delactosed whey, and whey, have partially corrected milk fat depression occurring from feeding high concentrate-low roughage rations. Hazzard et al. (17) reported a greater decrease in milk fat from cows fed a high-nitrogen whey product at a 10 per cent level than from the control high concentrate ration. The feed was partially refused by the cows due to an unpalatable nature of the whey product. They believed that the portion of feedstuff actually consumed was well utilized. More recent research, 1967, by Huber et al. (22) found that the addition of varying amounts of dried whole whey or partially delactosed whey to the concentrate ration tended to maintain fat tests while a depression in milk fat occurred on the control concentrate ration. Partially delactosed whey at the 20 per cent level gave some increase in fat test but no further response could be shown with a 30 per cent level. Both whey products gave a slightly decreased milk yield when

fed the concentrate containing a 60 per cent level of whey products. Huber et al. (21) in 1969, reported an increased milk fat synthesis from feeding restricted roughage rations containing whey or minerals. Various levels of partially delactosed whey were fed in the concentrate. The milk fat content increased as the concentration of partially delactosed whey was increased to 14 per cent, the highest level used in the experiment. Although the mineral rations gave the least fat depression, partially delactosed whey was almost as effective as minerals. They also found both rations causing a decreased content of linoleic acid in the milk fat but an increased content compared to the normal ration. Stearic and oleic acids increased on both rations with the mineral ration giving the greater increase for both acids. The delactosed whey gave no change in palmitic acid but gave an increase in the proportion of short and medium-chain fatty acids. Minerals in the ration decreased the amount of palmitic acid in the milk fat but no change was noticed in the short and medium-chain fatty acids.

Since 1962, there has been an increase in research on the phospholipids of milk fat, their distribution, and composition of their fatty acids (6, 7, 37, 38, 41, 42, 50). Jenness and Patton (23) stated that the phospholipids of milk are lacking the short-chain acids which are characteristic of the fatty acids found in milk fat. The principal fatty acids present in the phospholipid components are palmitic, stearic, oleic, and linoleic acids. They also indicated a greater proportion of unsaturation in milk phospholipids which causes oxidative deterioration.

EXPERIMENTAL PROCEDURES

Experimental Design

Thirty lactating dairy cows, about 60 days postpartum, were selected from the University Dairy herd and randomly assigned to one of five rations. Ration one, as a control, contained no whey products. Rations two through five contained, in addition to the control ration ingredients, dried whole whey, dried whey molasses, demineralized dried whey, or lactose respectively. The composition of the concentrate rations is listed in Table 1. The rations were adjusted as follows: the whey molasses diet contained the same amount of minerals as the dried whole whey ration, the demineralized whey diet contained the same amount of lactose as the dried whole whey ration, and the lactose diet contained the same amount of lactose as the dried whole whey ration.

Throughout the experiment there were six trials each involving five cows. Each trial consisted of a three-week standardization period followed by a six-week experimental period. The standardization ration contained alfalfa hay and corn silage fed ad libitum and the regular herd concentrate mix fed at the rate of 1 kg per 3 kg of milk produced. The experimental ration contained 2.3 kg of alfalfa hay as the only roughage and one of the five concentrate rations fed ad libitum.

Sample Preparation

Milk samples were collected once a week in sterile 250 ml bottles. Composite milk samples of 100 ml were prepared in the laboratory using

TABLE 1

Composition of the Experimental Concentrates

Ingredient	Rations					
	Standard	1	2	3	4	5
	%					
Ground Shelled Corn	51.25	79.4	66.5	7.6	69.2	67.5
Oats	34.00	--	--	--	--	--
Soybean Meal	11.25	12	11	9.6	10.5	14.2
Molasses	--	5	5	5	5	5
Urea	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium Phosphate	1.25	1.25	1.25	1.25	1.25	1.25
Trace Mineralized Salt	1.25	1.25	1.25	1.25	1.25	1.25
Dried Whole Whey[a]	--	--	14	--	--	--
Whey Molasses[b]	--	--	--	5.9	--	--
Demineralized Whey[c]	--	--	--	--	11.8	--
Lactose[a]	--	--	--	--	--	9.8

[a]Foremost Foods Company, San Francisco, California.

[b]Partially delactosed Spray Dried Whey Product, Valley Queen Cheese Factory, Incorporated, Milbank, South Dakota.

[c]Nutriteck 900, Foremost Foods Company, San Francisco, California.

60 per cent morning and 40 per cent evening milk to compensate for the volume produced at each milking.

The Roese-Gottlieb (3) extraction procedure was used to extract the lipids from the composite milk sample. All solvents used during the investigation were "Baker analyzed" reagent grade.[1] Diethyl ether and petroleum ether were redistilled in glass prior to usage. Fifty ml of the milk sample and the reagents (3) were mixed in a separatory funnel. The mixture was allowed to stand for 20 minutes until a biphasic system had formed. The lower phase containing essentially all the nonlipid components, was drained from the separatory funnel. The upper phase containing the lipid material was poured out of the top. A second extraction was done using appropriate volumes of solvents. The upper phase from the two extractions was combined. The lipid material was dried on a rotating evaporator under reduced water pressure at 40C, weighed, and taken up in diethyl ether for further analysis.

Phosphorus Determination

Phosphorus analysis was performed on the lipid extracts according to the procedure of Morrison (36). A weighed amount of lipid material, 20-40 mg, was transferred to a 30 ml micro-Kjeldahl flask, solvent evaporated under nitrogen, and digestion completed. The color was developed and the optical density read on a Bausch and Lomb Spectronic 20 spectrometer at 820 mμ.

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[1]Baker Analyzed Reagent, J. T. Baker Chemical Company, Phillipsburg, New Jersey.

Separation of the Lipid Extract into Neutral and Phospholipid Fractions

A modification of the silicic acid column procedure developed by Hirsch and Ahrens (20) was used for the separation of the milk fat samples. Mallinckrodt silicic acid, 100 mesh, labeled "suitable for chromatographic analysis by the method of Ramsey and Patterson" was used in the procedure. Approximately 300 grams of silicic acid and 700 ml of methanol were agitated together to create a suspension. The mixture was allowed to set until a definite layer of silicic acid could be seen. The methanol and finer particles were decanted off and discarded. The procedure was repeated with methanol, acetone, and diethyl ether. The washings remove the fine particles which could be washed from the column by the eluting solvents. The washed silicic acid was spread out in a glass dish and allowed to air dry for a few hours, then placed in an oven over night to complete the drying. Ten grams of washed silicic acid were slurried with diethyl ether into a glass column (2 cm x 42 cm) containing a glass wool plug. The column was washed with an additional 40 ml of diethyl ether.

Twenty five lipid samples were selected for separation into neutral and phospholipid fractions. For each ration, two samples during the standardization period and three samples during the experimental period were selected for analysis. These five samples were produced by one cow, whose performance during the experiment was judged to be representative of the six cows on the ration.

The selected samples 1.2-2.8 grams of milk fat were placed on the column with a disposable pipette and the sides of the column washed

several times to ensure that the entire sample was on the column. When the washings had just passed into the column, an additional 225 ml of diethyl ether were applied to the column to elute the neutral lipid fraction. This was followed by 175 ml of methanol to elute the phospholipid fraction. Both fractions were taken down nearly to dryness on a rotating evaporator under reduced water pressure at 40C. The neutral lipid fraction was taken up in diethyl ether and the phospholipid fraction was taken up in chloroform-methanol (2:1, *v/v*). The samples were stored in the refrigerator for future use.

Preparation and Analysis of Methyl Esters

Approximately 150 mg of lipid was transferred to a test tube and dried under a stream of nitrogen. The lipid samples were converted to methyl esters by a rapid technique according to Metcalfe et al. (33). The methyl esters were analyzed on a Varian Aerograph Series 1520 gas-liquid chromatograph equipped with dual-flame ionization detectors. The 3.05 m by 3.17 mm stainless steel column was packed with 10 per cent diethyleneglycol adipate polyester and 2 per cent phosphoric acid on Gas Chrom P 60/80 mesh.[1] Temperatures of operation were 240C for the injector and 250C for the detector. The column temperature was programmed from 70C to 180C at 4 degrees per minute to analyze butyric acid through linoleic acid. The conditions for the phospholipid methyl esters were the same except the column was operated isothermally at 180C. The peak area of each fatty acid was determined by triangulation.

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[1]Applied Science Laboratory, State College, Pennsylvania.

The quantitation of peak areas for the major components in a standard methyl ester mixture[1] indicated a relative error of less than 10 per cent.

Thin-Layer Chromatography

The basic thin-layer techniques of Stahl (51) were used for thick layer (0.4 mm) plate preparation with Silica Gel H[2]. The plates (20 cm x 20 cm) were air dried for three hours and then activated at 110C in an oven until used. The phospholipid samples of 100 μg each were spotted on the plates with a microsyringe. The plates were developed in chambers which had been lined with solvent-saturated filter paper and equilibrated for at least two hours. The solvent system reported by Rouser et al. (46) was used with modifications which included chloroform-methanol-water-28 per cent ammonia (130:70:10:0.5). Development of the plate required 50 minutes. Individual phospholipids were detected by the use of spray reagents. The specific phospholipid spray of Dittmer and Lester (10) was used to determine phospholipids containing phosphate esters. A spray containing a 0.2 per cent ninhydrin in ethanol was used for phospholipids containing free amino groups. The charring sulfuric acid-potassium dichromate reagent used by Privett et al. (44) was used to reveal the presence of all lipid material. Individual components were identified by comparing Rf values of the sample to those of a thin-layer standard.[1]

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[1]Applied Science Laboratory, State College, Pennsylvania.

[2]E. Merck A. G., Darmstadt, Germany.

RESULTS AND DISCUSSION

Total Fatty Acid Analysis

Presented in Table 2 is the gas chromatographic analysis of the total fatty acids in the milk fat. The standard ration, representing a normal ration fed during the standardization period, consists of the average values obtained from thirty cows during that period. The data represent the average of six cows on each ration during the experimental period. Slight differences were noticed between values in Table 2 for the standard ration and the values reported by Webb and Johnson (59). Metcalf et al. (33), as well as Webb and Johnson (59), reported a higher level of butyric acid than that value reported for the standard ration. This difference may be due to the difficulty with which butyric acid can be measured. Losses may have occurred even though a saturated salt solution was used to help overcome the loss of lower fatty acids due to the low volatility and greater water solubility.

The milk fat produced by cows fed the high grain control ration (ration 1) compared to that produced by cows on a normal ration (standard) contained more oleic and linoleic acids (100 per cent more linoleic), but the same proportions of butyric through palmitoleic acids. The other four rations; dried whole whey (ration 2), dried whey molasses (ration 3), demineralized dried whey (ration 4), and lactose (ration 5) when compared to the control ration caused increases in the fatty acids of the milk fat from butyric through palmitic acids. Similar results were obtained when compared to the standard ration

TABLE 2

Fatty Acid Composition of Milk Fat From Cows Fed High Grain-Limited Roughage Rations Containing Whey Products

Fatty Acid[a]	Rations					
	Standard	1	2	3	4	5
	%					
C4:0	1.7	1.6[b]	1.7[b]	1.9[b]	1.6[b]	1.9[b]
C6:0	2.3	2.1[d]	2.5[b,c]	2.5[b,c]	2.3[c,d]	2.9[b]
C8:0	1.5	1.4[d]	1.8[b,c]	1.7[c,d]	1.6[c,d]	1.9[b]
C10:0	3.3	3.2[d]	4.3[b]	4.0[c]	4.3[c]	4.7[b]
C12:0	3.5	3.8[d]	4.8[b]	4.4[c]	5.3[b,c]	5.3[b]
C14:0	11.6	12.0[c]	13.2[b]	12.8[b]	14.4[b]	13.6[b]
C16:0	30.4	28.3[c]	31.0[b,c]	29.9[b,c]	29.6[b,c]	30.1[b]
C16:1	1.8	1.9[b]	1.5[c]	1.6[b,c]	1.8[b,c]	1.6[c]
C18:0	12.1	9.3[b]	9.4[b]	10.2[b]	7.5[c]	8.4[b]
C18:1	29.7	32.4[b]	25.6[d,e]	27.5[c,d]	27.0[c]	25.7[e]
C18:2	2.1	4.2[b,c]	4.1[b,c]	3.5[c]	4.5[b]	4.1[b,c]
Saturated[f]	66.5	61.7	68.8	67.4	66.6	68.8
Unsaturated[g]	33.5	38.3	31.2	32.6	33.4	31.2

[a]Expressed as number of carbons: number of double bonds.

[bcde]Figures in the horizontal row for rations one through five followed by the same letter are not significantly different, $P < 0.05$, using Duncan's new multiple range test (52).

[f]Calculated as the sum of all the saturated fatty acids.

[g]Calculated as the sum of all the unsaturated fatty acids.

with the exception of palmitic and butyric acids. The milk fat from the demineralized whey ration decreased in butyric and palmitic acids. The milk fat from the cows fed the whey molasses and lactose rations showed slight decreases in palmitic acid. The standard ration produced greater amounts of palmitoleic, stearic, and oleic acids in the milk fat, than the four rations containing whey products. A lesser amount of linoleic acid was also produced. The control ration was 4.8 per cent more unsaturated than the standard ration with a corresponding decrease in saturation of fatty acids. The other four rations produced 7.3 per cent less unsaturation compared to the standard ration and 7.1 per cent less than the control ration.

The least squares analysis of variance was used to statistically analyze the fatty acid data obtained from the gas chromatographic scans. Values were entered as the difference between the average of the standardization period and each week of the experimental period. Appendix Table 2 indicates no significant difference for butyric and palmitic acids for weeks or rations. Only caproic, caprylic, capric, and linoleic acids contained significant differences between weeks. There was more difference between rations with only butyric, palmitic, and linoleic acids containing no significant differences. Stearic acid contained significant differences only at the 5 per cent level.

Duncan's new multiple-range test (52) was used to determine significant differences between rations one through five. The statistical results in Table 2 refers to the analysis of variance conducted on differences between the average of the standardization period and each

week of the experimental period. The values in Table 2 are only averages, therefore apparent discrepancies can be seen in statistical significance.

Butyric acid contains no significant changes between rations. The milk fat from the control ration contained significantly less capric, lauric, and myristic acids than the other four rations and significantly more oleic acid. The demineralized whey ration contained significantly less stearic acid than the other four rations. The only significant difference for palmitic acid is an increase from the control ration to the lactose ration. Linoleic acid was produced in significantly higher amounts for the demineralized whey ration compared to the whey molasses ration. There is no significant difference for any fatty acid when comparing the dried whole whey ration to the lactose ration. This is also true when comparing the whey molasses ration to the demineralized whey ration with the exception of stearic and linoleic acids which showed a decrease for stearic acid and an increase for linoleic acid on the demineralized whey ration. Except for capric, stearic, and oleic acids, there is also no difference between the dried whole whey ration and the demineralized whey ration. Decreases in capric and lauric acids are the only significant differences when comparing the dried whole whey ration to the whey molasses ration. Comparing the whey molasses ration to the lactose ration, caprylic, capric, and lauric acids are significantly increased and oleic acid is significantly decreased on the lactose ration. The last comparison is between the demineralized whey and the lactose rations.

The fatty acids which showed significant increases were caproic, caprylic, capric, and stearic acids with linoleic acid having a significant decrease on the lactose ration.

Analysis of Phospholipid Content

Preliminary investigations of the fatty acids indicate quite a noticeable change from feeding high grain rations containing whey products. For this reason, lipid phosphorus determinations were made on milk obtained from the last four cows on each of the five rations. Values for each week were averaged according to ration and analyzed statistically by the randomized complete block design analysis of variance. Highly significant differences ($P < 0.01$) were found between weeks and between rations as shown in Appendix Table 3. Duncan's new multiple-range test was also used to determine which rations were significantly different. Figure 1 illustrates that the milk from each ration increases in phospholipid content from the standardization period through the experimental period. The milk from the control and demineralized whey rations are significantly higher in phospholipids than the other three rations. Demineralized whey in the ration produced a sharp increase the first two weeks and again the last week of the experimental period with little change between the second and fifth weeks of the experimental period. This represents the largest increase of phospholipid content for the five rations. The control ration, with the next largest increase, produced large fluctuations during the experimental period. The dried whole whey and lactose rations were similar with a gradual increase up to the fourth week in the

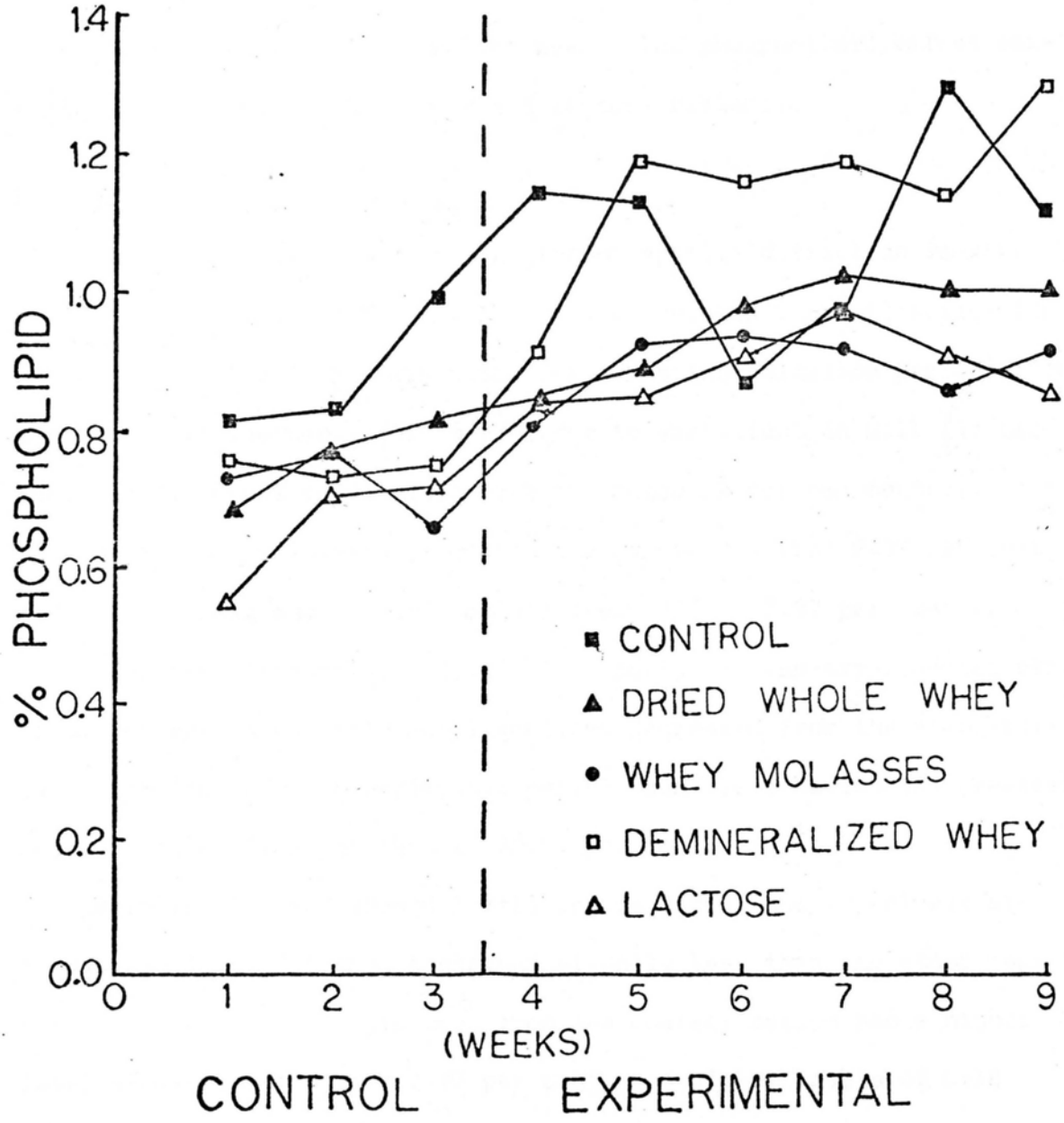

Figure 1. The phospholipid content of milk fat produced by cows fed high grain-limited roughage rations containing or lacking whey products. Control represents the standardization period. Values are the average of four cows and calculated as grams of lipid phosphorus per 100 grams milk fat x 25.

experimental period and then a decrease for the remainder of the period. The whey molasses ration produced a continual increase the first three weeks followed by a smaller decrease the fourth and fifth weeks and then another increase the last week. The phospholipid values were similar to the dried whole whey and lactose rations.

Phospholipid Fatty Acid Analysis

The fatty acid composition of the phospholipid fraction is shown in Table 3. The standardization period represents a normal ration fed to the cows. There are variations for the standardization periods from one ration to another. This may be due to variations in milk fat produced by different cows, since each cow acted as her own control.

On the control ration, butyric acid increased from 0.30 per cent to 1.77 per cent and linoleic acid increased from 8.97 per cent to 15.72 per cent from the standardization period to the experimental period. The remaining fatty acids analyzed decreased from the standardization period to the experimental period with oleic having the greatest decrease from 42.72 per cent to 38.28 per cent.

Palmitoleic acid showed little change during the experiment although the lactose ration contained slightly less than the other four rations. The milk fat produced from the control ration had a higher level of capric acid with 1.77 per cent, and higher levels of C:18 acids than all the other experimental rations except the lactose ration which produced more stearic and linoleic acids than the control. The control ration also produced less lauric, myristic, and palmitic acids than the other four rations with the exception of the lactose ration.

TABLE 3

Fatty Acid Composition of the Phospholipid Fraction from the Milk Fat of Cows Fed a High Grain-Limited Roughage Ration Containing Whey Products[a]

Fatty Acid[b]	Ration 1[c]		Ration 2		Ration 3		Ration 4		Ration 5	
	S[d]	E[e]	S	E	S	E	S	E	S	E
	%									
C10:0	0.3	1.8	0.7	0.9	1.4	1.1	1.1	0.8	0.6	0.9
C12:0	2.2	2.1	2.9	2.6	2.4	3.7	3.6	2.3	1.9	2.1
C14:0	7.5	7.3	10.4	9.0	7.0	13.2	12.1	9.4	5.9	5.6
Unknown	---	---	---	---	---	---	---	---	1.2	1.8
C16:0	24.2	21.3	26.2	26.3	25.2	28.9	28.8	29.8	21.1	20.6
C16:1	1.7	1.7	1.7	1.8	1.7	1.7	1.6	1.8	1.1	1.2
Unknown	---	---	---	---	---	---	---	---	1.8	3.2
C18:0	12.3	11.8	11.8	11.7	12.1	11.8	15.9	9.9	16.4	12.2
C18:1	42.7	38.3	37.2	34.4	43.3	33.1	31.6	35.0	37.9	35.2
C18:2	9.0	15.7	9.0	13.3	7.4	6.6	5.3	11.0	12.1	17.2
Saturated	46.6	44.3	52.0	50.6	48.1	58.6	61.5	52.2	46.0	41.4
Unsaturated	53.4	55.7	47.9	49.4	52.3	41.4	38.5	47.8	51.1	53.6

[a]Figures expressed are from one cow per ration.

[b]Expressed as number of carbons: number of double bonds.

[c]Average of two samples for each standardization period and three samples for each experimental period.

[d]Represents the standardization period.

[e]Represents the experimental period.

Less myristic and palmitic acids occurred from feeding lactose than from feeding a control ration containing no whey products. In comparing the rations containing whey products, the whey molasses ration produced more capric, lauric, and myristic acids and less oleic and linoleic acids, 33.10 per cent and 6.55 per cent respectively, than the other three rations. Feeding the demineralized whey ration produced 29.76 per cent palmitic and 9.86 per cent stearic acids which represents more palmitic acid but less stearic acid than the other three whey rations. Linoleic acid increased 50 per cent from the standardization to the experimental period on all but the whey molasses ration (ration 3). The degree of unsaturation increased for all rations except the whey molasses rations which gave a decrease in unsaturation from the standardization period to the experimental period. The demineralized whey ration had the largest increase of unsaturation. The lactose ration results are unusual because of the presence of two unknown peaks. This could be due to the fact that only one cow's milk was analyzed for each ration so it may have been that particular cow which produced unusual milk fat.

Thin-Layer Chromatography

Figure 2 represents a typical separation of the phospholipid components of milk fat. As shown in Figure 2, no visible differences between the major phospholipid components can be seen when comparing the standardization period to the experimental period.

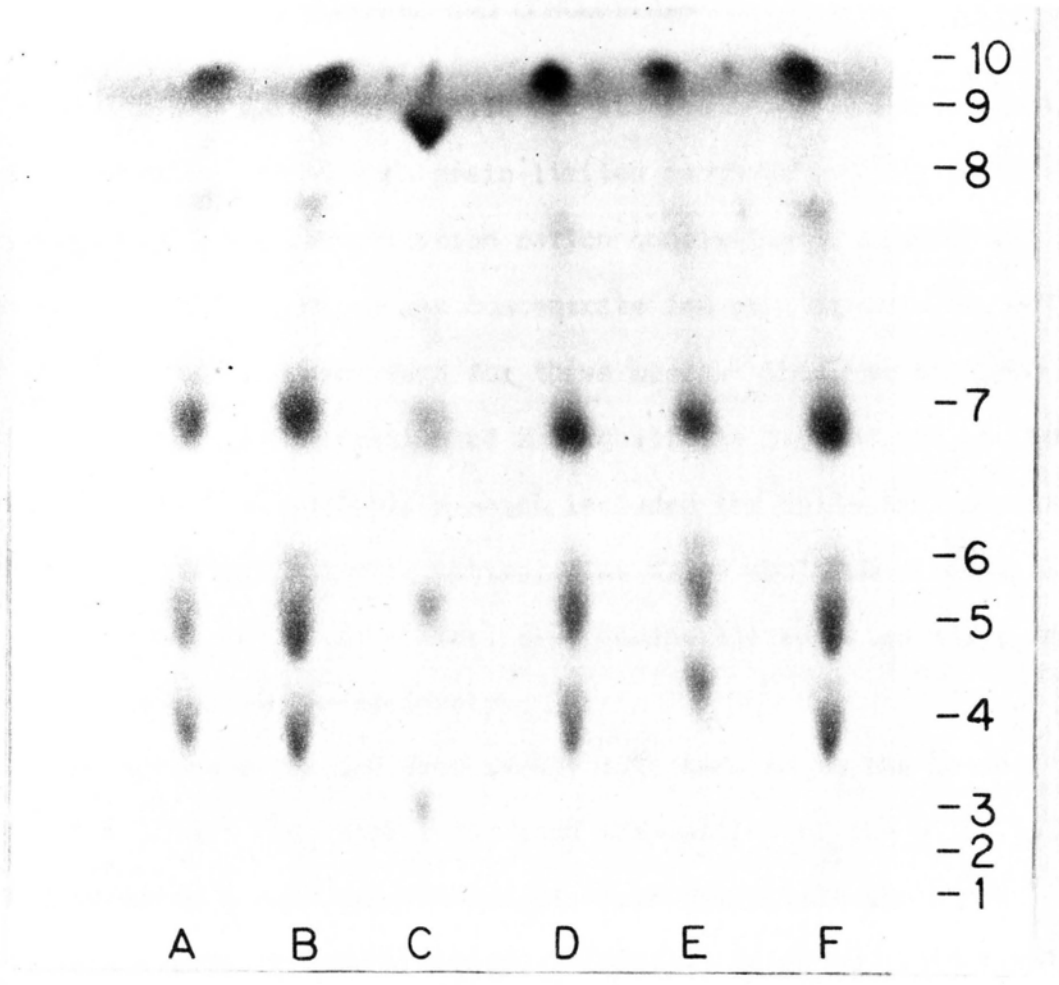

Figure 2. Thin-layer chromatographic separation of the polar lipids from milk fat. The chromatogram was prepared from Silica Gel H and developed with chloroform-methanol-water-28 per cent aqueous ammonia (130:70:10:0.5). Lipids were detected by sulfuric acid-potassium dichromate spray reagent (10). A, B, standardization period; C, thin-layer standard; D, E, F, experimental period; 1, origin; 2, lactose; 3, lyso-phosphatidyl choline; 4, sphingomyelin; 5, phosphatidyl choline; 6, phosphatidyl inositol and phosphatidyl serine; 7, phosphatidyl ethanolamine; 8, cerebroside; 9, cholesterol; 10, neutral lipids at the solvent front.

SUMMARY AND CONCLUSIONS

The lipid composition of milk was studied to determine changes that occur from feeding high grain-limited roughage rations containing whey products. A standardization ration consisting of alfalfa hay and corn silage fed ad libitum and concentrate fed at 1 kg per 3 kg milk was fed to 30 lactating dairy cows for three weeks. This was followed by a six-week experimental ration of 2.3 kg alfalfa hay and one of five concentrate rations ad libitum which included the following: control, containing no whey products; control plus dried whole whey; control plus dried whey molasses; control plus demineralized dried whey; and control plus lactose respectively.

Lipids were extracted from weekly milk samples by the Roese-Gottlieb method. The total fatty acid composition of the milk lipids were determined by gas chromatographic methods. Lipid phosphorus analysis was performed on the lipid extracts. Selected lipid samples was separated on a silicic acid column and the phospholipid fatty acid composition determined by gas chromatographic methods. Thin-layer chromatography was used to separate the phospholipid fraction into the major components.

The short-chain fatty acids of milk fat compared to a normal ration, increase when whey products are fed with high grain-limited roughage rations. Linoleic acid increased about 100 per cent on all high grain rations with the exception of the whey molasses ration which showed a 67 per cent increase. Whey molasses in the ration produced

similar fatty acid results as feeding a normal herd ration. The degree of unsaturation from feeding whey products in the ration was less than feeding a normal herd ration. Feeding a high concentrate ration containing no whey products produced milk fat higher in unsaturation than that of a normal herd ration. A high concentrate ration causes an increase in the phospholipid content of milk fat as compared to a normal herd ration. The phospholipid content of milk fat significantly increased on all experimental rations, with increases being significantly higher on the demineralized and control rations. This would indicate that some whey products such as dried whole whey, demineralized dried whey, or lactose help reduce the phospholipid content of milk fat produced by cows on high concentrate rations. The fatty acids of the phospholipid fraction are very similar to the total fatty acids of the milk fat when the control ration is compared to the other high concentrate rations containing whey products. The linoleic acid value for the whey molasses ration is an exception. Linoleic acid decreased from feeding a normal herd ration to a high concentrate ration containing whey molasses. Because of this difference, the degree of unsaturation also decreased when whey molasses were added to the ration. No visible change was seen in the polar lipid components. For this reason, further analyses of the components was believed not necessary.

The results of this experiment indicate that milk fat produced by cows on high grain-limited roughage could be more susceptible to oxidation due to the increased content of linoleic acid. Unpublished data of milk flavor evaluations from this University have shown a

decrease in oxidative stability of the milk from these cows. The higher phospholipid content would also indicate oxidative susceptibility. Results show that adding certain whey products to the high concentrate ration helps maintain fat production and produce milk fat which is more nearly like normal milk.

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APPENDIX

APPENDIX TABLE 1

Cows Used on Each Ration and the Analyses Performed on the Milk Fat

Ration	Cow Number	Phosphorus[a] Analysis	Phospholipid[a] Fatty Acid Analysis
1	2641	-	-
1	2868	-	+
1	2652	+	-
1	2870	+	-
1	2779	+	-
1	3112	+	-
2	2548	-	-
2	2889	-	-
2	2741	+	+
2	2771	+	-
2	2801	+	-
2	2807	+	-
3	2947	-	-
3	2695	-	-
3	2691	+	+
3	2561	+	-
3	2595	+	-
3	2854	+	-
4	2345	-	-
4	2419	-	-
4	2692	+	-
4	2729	+	+
4	2788	+	-
4	2836	+	-
5	2720	-	+
5	2764	-	-
5	2639	+	-
5	2345	+	-
5	2661	+	-
5	946	+	-

[a] + denotes the analysis was performed and

- denotes no analysis performed on the sample.

APPENDIX TABLE 2

A Summary of the Analysis of Variance on the Fatty Acid Data from Fat Produced on Rations Containing Whey Products

Fatty Acid	Error Mean Square	F Values	
		Weeks	Rations
C4:0	74.45	1.888	1.043
C6:0	87.34	5.246**	4.629**
C8:0	35.61	5.246**	5.829**
C10:0	15.14	3.384**	11.849**
C12:0	18.85	1.124	8.325**
C14:0	58.30	1.020	5.377**
C16:0	13.84	0.965	1.982
C16:1	43.62	0.578	3.485**
C18:0	95.23	1.105	2.514*
C18:1	32.01	0.560	13.460**
C18:2	26.04	3.547**	1.982

[a]Expressed as number of carbons: number of double bonds.
*= $P < 0.05$
**= $P < 0.01$

APPENDIX TABLE 3

Analysis of Variance on Phospholipid Content of Milk Fat

Source	DF	SS	MS	F
Total	44	1.290	0.029	
Weeks	8	0.662	0.083	9.22**
Ration	4	0.337	0.084	9.33**
Error	32	0.291	0.009	

DF = Degrees of freedom
SS = Sum of squares
MS = Mean square
F = F test
** = $P < 0.01$