ABSTRACT

Daily yields of milk, milk fat, phospholipids, and fatty acids were measured weekly during lactations of two groups of cows on a normal and restricted roughage diet. Milk yield was higher in normal cows during the initial 15 wk of lactation. Fat production decreased and was consistently lower in milk from cows on restricted roughage (from 1.2 to .4 in normal and from .8 to .3 kg/day for restricted). Phospholipid secretion decreased from an average 7 g to 3 g/day in both groups. The concentration of phospholipid in both milks fluctuated during lactation (20 to 30 mg/100 ml milk), but in milks from restricted cows it tended to increase with progress of lactation. Milk lipids from cows on restricted diets had higher phospholipid to fat ratio, (1.0 to 1.5 compared to .5 to .6 g phospholipid/100 g fat for milk from normal cows). Composition of the phospholipid classes changed slightly during lactation. Phosphatidylinositol changed most, increasing from 4 to 10 and 6 to 8% in normal and restricted milks, respectively. Fatty acids of short and medium chain lengths (C6 to C14) followed a typical quadratic regression in normal milks increasing from 10 to 30% of the total fatty acids during the first 25 wk of lactation. In restricted milk these fatty acids were already high (25%) within 2 wk and followed a linear regression with lactation. Both palmitic and stearic acid were lower in milk from restricted cows while oleic and linoleic acid were higher particularly after 10 wk of lactation.

INTRODUCTION

The effect of feeding high concentrate, lo fiber, or restricted roughage (RR) diets depressing milk fat secretion has been documented (1, 3, 5, 8, 9, 10, 23, 26, 27, 32, 34, 37, 39, 43). We have examined the constancy of the effects of a RR diet throughout a lactation on milk, fat, fatty acids, and phospholipid production. We were interested particularly in phospholipid (PL) secretion in relation to fat in milk.

Phospholipids, being amphipathic molecules, perform many important functions in relation to synthesis, secretion, stabilization, and processing of milk fat. Thus, lecithin has been implicated in the formation of intracellular fat droplets (15, 30). Phospholipoproteins of the cell membrane envelop the fat droplet in secretion and function as surfactant in maintaining the milk fat in suspension (29), and they may protect the fat from lipases in milk. In addition, PL affect several quality attributes, technological processes, and applications of dairy products (6, 17, 24). Furthermore PL, via autoxidation, are a primary source of off-flavors in those dairy foods possessing mild flavors, e.g. butter, milk, and dairy powders (18).

Because of their importance and the lack of such data we have monitored the concentration and composition of PL in normal milk during a lactation cycle. Concurrently, to determine if any relationship existed between fat and PL secretion, we studied milk from cows on a restricted roughage diet.

MATERIALS AND METHODS

Six Holstein cows, of similar age that calved in November, were randomly assigned to two groups of three cows each. One group was given normal (N) hay ad libitum, plus a pelleted concentrate at 1 kg per 4 kg of milk; the other group (RR) received hay restricted to less than 25% of dry matter intake plus pelleted concentrate fed to appetite. The composition of these diets was determined. Daily consumption and milk yields were monitored throughout lactation (40, 41). The total fat in samples from individual cows was determined at weekly

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MILK PHOSPHOLIPIDS

Milk lipids were extracted by the procedure of Folch et al. (11). The fatty acid composition was determined by gas liquid chromatography (19). The PL were fractionated and quantified by the method of Parsons and Patton (28). The analytical data obtained at weekly intervals for components of milk from cows in each treatment were averaged and covariance of averaged data was analyzed to determine effects of treatment and stage of lactation on yield and lipid components. Regression analyses were used to fit the data to a curve, and analysis of variance was employed to determine which equation was most applicable. Letters are used to denote milk or its components from the cows on normal (N) and restricted roughage (RR) diet.

**RESULTS**

Averaged milk yields from cows within the same treatments were plotted against stage of lactation (Fig. 1). The cows on the conventional control diet produced more milk during the initial 15 wk of lactation, and thereafter yields from both groups were comparable. The production data and trends for normal cows were consistent with earlier reports (4, 12, 36, 38).

The yield of fat decreased progressively with lactation, and fat production by cows on RR was significantly lower than that from normal cows at all stages of lactation (Fig. 1). In both groups the concentration of fat (g/100 ml) increased with advancing lactation. These data confirm earlier reports (5, 8, 27, 34, 35, 37, 43) and show that the RR diet exerted its fat depressing effect throughout lactation indicating a stable but reversible metabolic adaptation to it.

Phospholipid secretion was apparently more variable than the other components in weekly fluctuations. Yield of PL decreased from an average of 7 to 4 g/day in milk from cows on normal diet whereas average PL in milk from cows on RR diets remained between 5 to 6 g/day (Fig. 1). The actual concentration of PL in milk (g/100 ml) from both groups of animals

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**FIG. 1.** Regression showing average lactational trends in production of milk, milk fat, and phospholipids by cows on a normal (+) and restricted roughage (○) ration. The respective equations for these regressions were:

\[ (+) y = 22.12 + 4.31W - .503W^2 + .0185W^3 - .000226W^4 \]

\[ (o) y = 19.55 + 2.53W - .255W - .255W^2 + .00816W^3 - .000089W^4 \]

\[ (+) y = 1.197 - .0401W + .000566W^2 \]

\[ (o) y = .736 - .0226W + .000314W^2 \]

\[ (+) y = 6.652 + .0612W - .00578W^2 \]

\[ (o) y = 7.0876 - .152W + .00209W^2 \]

for daily milk production, fat, and phospholipid yield from cows on normal (+) and restricted roughage (○) diet.

**FIG. 2.** The concentration of phospholipids in milk and milk fat during the lactation of cows on normal (+) and restricted roughage (○) diet.
tended to increase though not in a regular fashion (Fig. 2). In general, except for wk 6 through wk 12, PL were higher in the milk from RR cows.

To ascertain if there was a relationship between PL and fat secretion, ratios between these during lactation were plotted (Fig. 2). The ratio showed a cyclic pattern in the normal milk while ratios in RR milks were more scattered though they were higher most times.

The relative concentration (percentage distribution) of the PL classes remained constant throughout lactation in normal cows with the exception of phosphatidylinositol (Pl) which progressively increased with lactation in both groups (Fig. 3). Milk PL classes from cows on RR diets showed some minor changes. Thus, the choline containing lipids phosphatidylcholine (Pc) and sphingomyelin (Sp) decreased while phosphatidylethanolamine (Pe) and Pl increased.

The fatty acid compositions of the total lipids were different in the two groups. The aggregate short and medium chain fatty acids showed a typical quadratic regression with the progress of lactation in the normal cows, in contrast to those from the RR cows, which were best fit to a linear regression (Fig. 4).

The concentration of palmitic acid followed a quadratic and a linear (negative) regression for the N and RR diets, respectively, and after the

![Graph showing changes in phospholipid classes during lactation](image)

**FIG. 3.** Variation in phospholipid classes during lactation of cows on normal (solid line) and restricted roughage (broken line) diets: Phosphatidylinositol (Pl), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and sphingomyelin (Sph). Equations for these regression lines for normal and restricted roughage diets are:

- PE: \( y = 37.2 - 0.078W; y = 31.2 + 0.364W - 0.007W^2 \) for PE;
- PC: \( y = 33.0; y = 37.7 - 0.184W \) for PC;
- Sph: \( y = 23.00 - 0.066W, y = 25.13 - 0.516 + 0.01W^2 \) for Sph; and
- Pl: \( y = 6.23 + 0.063W, y = 4.02 + 0.150W \) for Pl.

![Graph showing changes in milk fatty acids during lactation](image)

**FIG. 4.** Changes in composition of milk fatty acids during lactation of cows on normal (solid line) and restricted roughage (broken line) diets. C6 through C14 denote those short and medium chain fatty acids synthesized in mammary gland; C18:2 linoleic acid. Equations for the regression lines of milk fatty acids from normal and restricted roughage respectively are:

- C18:2: \( y = 4.39 - 0.055W; y = 2.91 + 0.150W - 0.003W^2 \) for C18:2;
- C4 to C14: \( y = 11.0 + 1.22W - 0.023W^2; y = 23.9 \) for C4 to C14;
- Palmitic: \( y = 24.4 + 0.503W - 0.0119W^2; y = 26.93 - 0.084W \) for palmitic acid;
- Stearic: \( y = 14.1 - 0.328W + 0.0083W^2; y = 7.65 - 0.163W + 0.0076W^2 \) for stearic acid; and
- Oleic: \( y = 35.12 - 1.10W + 0.0245W^2; y = 31.75 - 0.18W + 0.0037W^2 \) for oleic acid.
5th wk with palmitic acid was significantly lower in the milk from cows on RR (Fig. 4). Stearic acid was significantly lower in the RR milk though the disparity diminished with time. Oleic acid was lower in normal milks after the 5 wk of lactation. In both treatments oleic acid decreased, though to different extents, until wk 25 after which relative concentrations increased.

Linoleic acid progressively decreased in normal milk from (4 to 2%) while that in RR milk increased (Fig. 4). Linoleic acid was negligible in normal milk but increased in RR milks. Some correlation coefficients between these components are in Table 1.

**DISCUSSION**

Data concerning milk yield and fat production by the normal cows are in accordance with reports (4, 12, 36, 38). The concentration of the short and medium chain fatty acids in normal milk followed a quadratic pattern almost identical to that reported by Dimick and Harner (7) while fatty acids in general behaved similarly to trends recorded by Stull et al. (36).

Cows on the RR diet produced less fat throughout lactation thereby confirming previous short term studies concerning the effects of low roughage diets (1, 3, 5, 8, 9, 26, 27, 34, 35). The concentration of short and medium chain fatty acids became relatively high rapidly after parturition indicating the availability of their precursors, acetate and β-hydroxybutyrate, to the mammary tissue and perhaps the absence of inhibition of mammary fatty acid synthetase by an excessive influx of unesterified, long chain fatty acids, which conceivably occurs in normal cows (33). The constant percentage of these short and medium chain fatty acids in milk from cows on low roughage, high concentrate diets was in keeping with earlier data from short term feeding and field studies (3, 23).

Patterns of long chain fatty acids, palmitic and stearic acid, were generally consistent with earlier analyses of these acids from cows on restricted roughage diet (1, 3, 8, 9). Concentration of these was lower in fat depressed milks. Askew et al. (1) showed a linear relationship between fat depression and the decrease in these two components. Thus, less saturated fatty acids in RR may have limited milk fat synthesis. This is consistent with observations and conclusions of Baldwin et al. (3), Askew et al. (1), and Opstvedt and co-workers (26, 27). In cows receiving low roughage diets long chain fatty acids may be preferentially utilized in deposition of adipose triglycerides rather than being used for milk fat synthesis. This is also in keeping with behavior of the relevant biosynthetic enzymes of mammary tissue which apparently are more active with long chain fatty acids (2, 20, 21).

Oleic, linoleic, and linolenic acid were higher in the RR samples after the initial 5 wk. This trend is also consonant with previous reports (1, 3, 5, 8, 23, 27).

While the effects of RR diet are exercised throughout lactation on fat yields and fatty acid composition, the response, particularly fatty acid composition, varies with the progress of lactation. This may explain partly the varying responses of cows to fat depressing diets reported by several authors.

Though the total quantity of PL secreted per

### Table 1: Correlation coefficient matrix showing strength of relationships between milk compositions measured during a lactation cycle of cows on a conventional (N) and a restricted roughage (RR) diet.

<table>
<thead>
<tr>
<th>Week of lactation</th>
<th>Milk yield (kg/day)</th>
<th>Fat yield (kg/day)</th>
<th>Phospholipid yield (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>RR</td>
<td>N</td>
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<tr>
<td>Week of lactation</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Milk yield (kg/day)</td>
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<tr>
<td>N</td>
<td>.93</td>
<td>.93</td>
<td>.81</td>
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<tr>
<td>RR</td>
<td>-.91</td>
<td>.80</td>
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<td>Fat yield (kg/day)</td>
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<td>N</td>
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<td>RR</td>
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<td>.71</td>
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<td>Phospholipid yield (g/day)</td>
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<tr>
<td>N</td>
<td>-.78</td>
<td>.81</td>
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<tr>
<td>RR</td>
<td>-.45</td>
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day decreased, the concentration of PL in milk increased and the PL to fat ratio tended to remain constant over the lactation period. Concentrations of PL in normal milk were mostly within the ranges reported for individual milk samples, i.e. 25 to 40 mg PL/100 ml milk (13, 15, 22, 28, 29, 31) as are the PL to fat ratios .2 to 1.0 g PL/100 g milk fat (22).

In general PL were higher in RR milks. This may be due to greater numbers of smaller fat droplets in RR milks (6) though there was no direct determination of this in our study.

Phospholipids of milk occur mostly in the fat globule membrane which is acquired during secretion (29). The average size of fat droplets in milk decrease from an average diameter of 4 to 2.5 to 3μ during the lactation cycle (6, 14, 42). The concentration of fat in the milk increases during this time. These two phenomena result in a significant increase in total surface area of milk fat globules with advancing lactation. Hence, presumably, there is an enhanced requirement for PL (phospholipoproteins) to enrobe this fat. This may partly explain the increase in PL, particularly noticeable in RR milk, toward the end of lactation. Estimating, at minimum, a doubling of the surface area of milk fat droplets (i.e. average fat droplet size decreasing from 4 to 2μ, with concomitant increase in fat concentration) suggests that PL for fat membrane material is scarce in milk from cows in late lactation because its concentration increases by only 30% during this stage. Thus, such fat droplets may have PL deficient, or defective membranes, and such milks may be more susceptible to hydrolytic rancidity and churning during storage and transport. Of course the possibility of increasing amounts of cytoplasmic materials being secreted into late lactation milks must also be considered.

These observations indicate the need to study the relationships between fat globule size, their phospholipid content, and susceptibility of fat to lipolysis in milk from normal cows and cows in late lactation.

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REFERENCES


