Effect of Triundecanoin upon Lipid Metabolism in the Cow

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ABSTRACT

The effect of feeding an odd-numbered carbon triglyceride, triundecanoin, upon lactation in the cow was studied. This fat appeared to be subject to considerable degradation in the rumen. There was no indication, however, that it yielded any increase in relative propionate concentration. A large amount of the odd-numbered carbon triglyceride was rejected by the animals, with a general decrease in feed consumption and consequent reduced milk yield. Fat production was unchanged. Some undecanoate passed into the milk but only at a low rate. There was a large difference in animal response. Feeding triundecanoin protected from rumen degradation led to a much greater transfer of undecanoate to the milk, prevented a significant decline in feed consumption and milk yield, but did not affect the milk composition appreciably. Smaller increases were observed in the amounts of other odd-numbered carbon fatty acids in the milk.

INTRODUCTION

The fatty acids of cow's milk are derived both from synthesis in the udder and from dietary sources, the former serving as a source for the short chain fatty acids and the latter for the long chain acids (1). The C18 acids in particular are derived directly or indirectly from dietary sources, though the bulk of the polyunsaturated C18 acids in the diet are hydrogenated in the rumen to C18:1 or C18:0. The ratio of acetate and propionate formed in the rumen has a considerable bearing not only upon the yield of milk fat but also upon the relative amounts of the lower fatty acids present in the milk. Increased acetate promotes an increase in both fat yield and amount of the lower fatty acids synthesized in the udder, whereas decreased acetate and increased propionate bring about the reverse (2). With odd-numbered carbon fatty acids, propionate results as the end-product of metabolism, and, hence, it seems conceivable that the feeding of such acids to the ruminant might bring about the same results as those obtained by feeding dietary constituents that promote the synthesis of propionate in the rumen. The difference, of course, is that the degradation of the odd-numbered carbon fatty acids also will yield two carbon fragments as well, the exact number of which will depend upon the length of the chain, so that the net effect of feeding such a compound might be difficult to predict.

Small amounts of odd-numbered carbon fatty acids, in particular C9:0 and higher, derived from the condensation of propionate with acetate groups, occur normally in milk fat (3). Infusion of n-pentadecanoate into the udders of goats led to its transfer in large amounts to milk fat (4). Emulsions of tripelargonin infused into the jugular vein of lactating cows increased the yields of C9:0, accompanied by decreased amounts of even-numbered carbon fatty acids (5). However, fatty acids shorter than C10:0, when absorbed from the intestinal tract, are transported by way of the portal venous system as free fatty acids, rather than as triglyceride elements of chylomicrons, as are the longer chain fatty acids; they go directly to the liver where they are virtually completely oxidized. Thus, feeding the short chain acids would not be an efficient way of transferring these acids to the tissues (6). On the other hand, feeding triundecanoin to rats and dogs led to deposition of C11:0 in their adipose tissue (7,8). Thus, the feeding of C11:0 or higher odd-numbered carbon fatty acids might be expected to yield increased amounts of these acids in ruminant milk fat if they escaped degradation by the ruminal microflora.

To test the possibilities mentioned above, triundecanoin was administered to lactating dairy cows in the present experiments.

EXPERIMENTAL PROCEDURES

In experiment 1, two cows in the second-third month of lactation were fed a basal ration of grain, silage, and alfalfa hay pellets; the amounts of grain fed were based upon milk production. The experiment consisted of two test periods of 5 days each. In the first test, one cow received triundecanoin and the other corn oil; in the second, the treatments were reversed. In each case, the fat was substituted for an equal wt of grain and mixed in with the remainder of the grain. Triundecanoin was offered at the ratyof 966 g/day in the first test and 1034 in the second (5.1 and 5.4%, respectively, of the total ration). Corn oil was given in the same amounts; however, it constituted 4.7 and 5.9%, respectively, of the total ration because the amounts of silage offered were changed slightly between the 2 test periods. There were 3 control periods: (A) the 7 days prior to the first test period, (B) the 9 days between the two test periods, and (C) the 9 days after the second test period.

In experiment 2, melted triundecanoin (1484 g) was infused by cannula into the abomasum of a cow in the ninth month of lactation in a single infusion at the rate of 6.5 ml/min.

In experiment 3, a cow in the fourth and a cow in the fourteenth month of lactation were fed a basal ration of grain and alfalfa hay, the amounts offered depending upon milk production. There were two 5 day control periods, one before and one after the test feeding. Test feeding lasted for 4 days. Each cow received triundecanoin encapsulated with sodium caseinate that had been treated with formaldehyde as a protective agent against possible degradation in the rumen (method of encapsulation described in [9]). The protected particles were substituted for grain as in experiment 1. They were fed in amounts to supply 800 g/day encapsulated triundecanoin; this represented 496.5 g triundecanoin, or 2.8 and 3.1% total ration for the 2 cows.

The triundecanoin used in these experiments was a triglyceride of 99+% purity, with a free fatty acid content of 0.03%, and a saponification value of 280. It contained C11:0 as 98.94% total fatty acids present. It was prepared from undecanoic acid derived from castor oil. (Drew Chemical Co., Boonton, N.J.)

In all experiments, feed intake was measured daily and milk yield determined for each milking. Milk samples were collected twice daily. Determinations were made for fat, protein, and solids-not-fat (SNF) on daily composites, except for experiment 1 in which samples from only the last 2 days of a period were analyzed.

Fatty acid composition (C4:0 -C18:3) was determined on daily composites in experiment 1 and on samples from each milking in experiments 2 and 3. Fat was extracted

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TABLE I

Feed Intake, Milk Production, and Composition of Milk (Experiment 1)

Observation	Control[a]	Triundecanoin[b]	Control[a]	Corn oil[b]
Average feed intake (kg/day)[c]	18.1	12.8**	18.2	18.1
Average milk production (kg/day)	23.9	19.9**	24.5	24.0
Composition of milk:				
Fat (%)	2.3	3.3	3.0	3.6
(kg/day)	0.67	0.67	0.74	0.86*
Protein (%)	2.92	2.70	2.92	2.80
(kg/day)	0.70	0.54**	0.72	0.67**
Solids-not-fat (%)	8.58	8.43	8.56	8.68
(kg/day)	2.05	1.68**	2.10	2.09
Cholesterol (mg/100 ml)	11.8	14.1	11.3	11.3

[a]Results for control periods before and immediately after the test treatment were included together.

[b]* = difference between control and treatment group significant at 5% level; ** = difference significant at 1% level; no asterisk indicates no statistically significant difference.

[c]Weight of silage expressed on an air-dry basis.

with chloroform-methanol (2:1) essentially by the method of Storry and Millard (10). Methyl esters were formed by the procedure of Christopherson and Glass (11) in sealed vials. The esters were analyzed by programed (65-180 C) gas liquid chromatography (GLC) on 10% EGSS-X on Gas-Chrom P (100-200 mesh) (Applied Science Laboratories, State College, Pa.) in a 0.6 cm x 183 cm glass column with a model 7620 Hewlett-Packard gas chromatograph. Milk cholesterol was determined on daily composites by the method of Sobel and Mayer (12).

In experiment 1, rumen fluid samples, blood samples, and feces grab samples were collected at the end of each period. Rumen samples were obtained by inserting large bore tubing through the mouth and down the esophagus into the rumen and aspirating about 1 liter of fluid and ingesta through the tubing with a portable vacuum pump. The collected material was filtered through several layers of cheesecloth. A sample of the filtrate was analyzed for volatile fatty acids by GLC at 150 C on 10% free fatty acid phase on Chromosorb W, (Supelco, Bellefonte, Pa.) in a 0.63 cm x 183 cm glass column with a model 900 Perkin-Elmer gas chromatograph. Blood samples were taken by jugular venapuncture. They were analyzed for fatty acids (as described above for milk samples), plasma cholesterol (12), plasma triglycerides (13), and nonesterified fatty acids (14). Feces samples were analyzed for fat content (ether extract, using Goldfisch extraction apparatus) and fatty acid composition.

In experiments 1 and 3, the results given in the tables represent averages of two cows. Statistical calculations are based upon comparisons between control and experimental periods after first averaging the values obtained for each cow in every instance. Tests of significance are based upon Student's t-test. Because there appeared to be a lag of ca. 1 day before the effects of the experimental treatment influenced the milk composition, values for the experimental period for milk fatty acids are based upon the second day of the period through the first day following the end of treatment. Values for the first day of the experimental period were not included in any average.

RESULTS AND DISCUSSION

When triundecanoin was fed, there was an average drop in daily feed intake of 29% (Table I). However, the two cows varied, not so much in total decrease (25 and 34%) but in the nature of the decrease. During the first 3 days, both cows failted to consume about 25% grain offered; during the latter 2 days, however, cow A refused ca. 30% and cow B refused 73%. Because the grain constituent contained the triundecanoin, obviously, cow A had a greater intake of the substance. Some of the refusal possibly could be attributed to the increased energy content of the triundecanoin ration, but this seems unlikely since feed intake did not decline on the corn oil ration which contained about the same amount of substituted fat. Several more likely explanations include an appetite depressing effect attributable to the taste of the triundecanoin, an indirect action on an appetite center in the hypothalamus, or a metabolic result brought about by an inhibiting substance formed in the rumen or blood.

Milk product also went down (17%) when triundecanoin was fed (Table I), though not as much as feed intake. The decrease was undoubtedly due to the decline in feed intake, with which it was highly correlated ($r = 0.71$, $P < 0.01$). Riddett, et al., (15) observed that when cows were changed from a full production to a one-half production ration, milk yield fell ca. 25%. The lowering of milk production observed here bore about the same ratio to decrease in feed intake. Milk production did not change with corn oil feeding.

In spite of the reduced yield when triundecanoin was fed, average amount of fat produced was the same (Table I). The difference came in the yield of protein and other SNF in milk. The lowered milk yield with decreased feed intake observed by Riddett, et al., (15) also was attributable to a large reduction by Riddett, et al., (15) also was attributable to a large reduction in the SNF component, with no consistent effect upon the fat content. Fat yield went up slightly, whereas protein went down slightly when corn oil was fed. With vegetable oils, Allen and Fitch (16) observed either an increase in fat test or little change during a 5 day feeding period; but, as the experiment progressed beyond that time, both fat test and yield decreased sharply with both corn oil and soybean oil. Neither triundecanoin nor corn oil feeding had a significant effect upon the cholesterol content of milk (Table I).

Undecanoic acid (C11:0) increased most sharply (23x) of all the fatty acids in milk when triundecanoin was fed. However, not surprisingly, because their intake varied, the two cows differed considerably in the extent to which this odd-numbered carbon acid rose in their milk. With cow A, which consumed the most triundecanoin, C11:0 increased 66x; with cow B, only 8x. In the milk of cow A, C11:0 rose to 6.1% fatty acids by the end of the period. In the milk of cow B, it reached only 1.6%. This level was attained on the second day after feeding began and declined steadily thereafter. The differences between the cows are greater than might be expected from intake differences and suggest a possible metabolic interference preventing transfer of the C11:0 to the milk most likely involving a greater catabolism of the fatty acid in the rumen. With cow A, 1.5% C11:0 fed

TABLE II

Yields of Fatty Acids in Milk Fat (Experiment 1).

Fatty acid	Control[a]	Triundecanoin[b]	Control[a]	Corn oil[b]
		(g/day)		
C11:0	0.8[c]	16.3**	0.5	0.5
C13:0	1.0	0.7**	0.7	0.4
C15:0	8.5	4.5**	6.8	4.6**
C17:0	4.1	4.1	4.2	2.8*
C 8:0	6.4	5.0**	6.8	8.1
C10:0	16.1	9.9**	18.7	18.2
C12:0	20.2	11.2**	24.5	21.4
C14:0	71.0	43.4**	80.4	71.5
C14:1	9.6	4.1**	9.8	6.3**
C16:0	203.9	159.1**	210.6	201.2
C16:1	21.0	19.6	21.0	18.1
C18:0	41.0	57.6**	51.3	72.4**
C18:1	191.5	240.5**	197.7	274.9**
C18:2	21.6	18.3	23.1	33.1**
C18:3	7.8	6.8	10.0	20.6**

[a]Results for control periods before and immediately after the test treatment were included together.

[b]* = difference between control and treatment group significant at 5% level; ** = difference significant at 1% level; no asterisk indicates no statistically significant difference.

[c]Using only preexperimental controls, because C11:0 remained slightly elevated well into the post experimental period.

was recovered in the milk; with cow B, only 0.8%. None of the other odd-numbered carbon fatty acids increased in the milk of either cow.

Shifts in the relative composition of the even-numbered carbon fatty acids in milk also were observed. C18:0 and C18:1 incrreased whereas C16:0 and lower fatty acids significantly decreased.

Changes in percentage values were reflected in similar changes in the actual amounts of the fatty acids produced (Table II). It is not easy to see why the yields of the C18 acids should be increased by the feeding of triundecanoin, because it seems unlikely that the breakdown of C11:0 would lead to the formation of C18 acids, which usually are not formed in the rumen but transferred from the food (1). However, ruminants use fats less efficiently than nonruminants (17), and oxidative degradation of the C18 acids, though small, apparently takes place in the rumen to some extent (18).

The triundecanoin may have disturbed the rumen metabolism of these cows sufficiently to the extent that less of the C18 acids were degraded there. If this happened, greater amounts then would be available for transfer to the milk. There would appear, however, to have been no interference with the customary hydrogenation that occurs in the rumen, since the fatty acids of the milk fat contained relatively large proportions of C18:0 and C18:1 and relatively small amounts of the polyunsaturated fatty acids, in contrast to the relatively large proportions of C18:2 and C18:3 that occur in grain and hay fed to cows (19).

Feeding corn oil to the cows brought about similar changes in the relative amounts of the even-numbered carbon fatty acids in milk—decreased C16:0 and lower acids and increased C18 acids. In most cases, the absolute amounts of the lower acids did not decrease significantly (Table II). However, the yields of C18 acids increased signifcantly. Presumably, in short term feeding of unsaturated vegetable oils, the milk fat yield tends to increase because of transfer to the milk of C18 acids from the diet after being mostly hydrogenated in the rumen, whereas the decrease in the short chain fatty acids, due to an effect of the oils upon the rumen metabolism, begins more slowly. As the experiment continues, the amounts of these acids synthesized decrease much more sharply along with an attendant reduction in total milk fat yield.

The other samples in experiment 1 were too small in number to permit any meaningful statistical comparison but are interesting to examine. Rumen fluid comparisons gave little indication of any difference in the relative distribution of the volatile fatty acids between control and triundecanoin feeding. There was certainly no relative increase in propionic acid production. There was a suggestion of a decrease in total volatile fatty acid production upon triundecanoin feeding. However, the decline occurred only in cow B, in which production dropped from 7.5 to 2.8 meq/100 ml. Because cow B rejected the most triundecanoin, this decrease suggests further that the refusal was associated with a disturbance in rumen metabolism. Rumen pH was not affected. Corn oil feeding had no noticeable effect within the feeding period upon any rumen characteristic measured.

Storry, et. al., (20) found a reduced cholesterol concentration in the blood plasma in both of two cows tested and an accompanying lower triglyceride level in one of them when a supplement of fat was removed from the diet. Storry, et al., (5) observed increased cholesterol and triglyceride concentrations in blood plasma when a number of synthetic triglycerides were infused intravenously into lactating cows. With the cows on the odd-numbered carbon tripelargonin, however, only the triglyceride values were elevated. There was no indication in the present experiments that the triglyceride concentration in blood plasma was affected by the feeding of either triundecanoin or corn oil. Cholesterol and nonesterified fatty acids appear higher

TABLE III

Effects of Infusion[a] of Triundecanoin on Composition of Milk (Experiment 2)

Observation	Average days -1 and 0	Day 1 am	Day 1 pm	Day 2 am	Day 2 pm	Day 3 am	Day 3 pm	Average days 4 to 6
Milk yield (kg/day)	8.2	5.6		6.7		8.2		7.6
Fat (%)	4.6	4.8		4.7		4.5		4.1
Fat (g/day)	377.2	268.8		315.2		367.0		311.6
Protein (%)	2.9	2.9		---		---		3.1
Solids-not-fat (%)	8.7	8.6		8.6		8.6		8.6
Fatty acid (wt % of total)								
$C_{11:0}$	---	7.8	4.2	1.5	0.7	0.3	0.1	---
$C_{13:0}$	0.1	0.4	0.4	0.2	0.2	0.1	0.2	0.1
$C_{15:0}$	0.8	1.2	1.1	0.9	1.1	0.9	1.0	0.8
$C_{16:0}$	26.6	25.1	27.2	28.4	24.5	28.9	27.9	28.0
$C_{18:0}$	14.9	13.0	12.2	15.4	19.9	16.4	14.2	14.6
$C_{18:1}$	29.8	25.2	25.2	26.6	28.7	26.0	27.4	29.6

[a]Infusion on day 0 within 5-1/2 hr period encompassing the day 0 pm milking, which showed no effect of the treatment.

when triundecanoin was fed. The higher levels occurred in only one of the two cows, however, a different cow in each case. Thus, these differences cannot be regarded as necessarily meaningful. Plasma cholesterol appeared higher when corn oil was fed but again in only one of the two cows.

Detectable amounts of C11:0 appeared in the blood plasma and feces when triundecanoin was fed. Much higher levels appeared in cow A (1.2% total fatty acids in plasma and 4.1% in feces) than in cow B (0.1% in plasma and 1.2% in feces). Compared with results from control periods (2.3-3.1%), fat represented a greater proportion (4.8%) of feces when corn oil was fed and a lower amount (2.0%) when triundecanoin was given. However, the latter effect was noticeable only in cow B, in which the fecal fat dropped to 1.5%.

Because triundecanoin seemingly was broken down in the rumen to a large extent, attended by adverse effects upon feed intake and milk yield, it seemed desirable to determine the results of bypassing the rumen. In experiment 2 (Table III), the odd-numbered carbon triglyceride was infused into the abomasum of a cannulated cow. The dose given was ca. 1.5 x as large as the daily amount offered/cow and nearly 2.5 x as much as the average daily amount consumed in experiment 1. Milk production was reduced by one-third on the day after infusion but returned to normal by the third day. This drop was sharper than that observed during experiment 1 but was not due to triundecanoin per se because infusion of corn oil to the same cow 1 week later led to a similar decrease. The relative composition of the milk essentially was unchanged, and, thus, the amounts of fat, protein, and SNF were all lower during the first 2 days following infusion. The relative amount of C11:0 in the milk rose to 7.8% in the first milking after infusion was completed, gradually decreasing in each milking after that until the fourth day when detectable amounts were no longer present. Efficiency of transfer to the milk was 1.4%, or about the same order of magnitude as that of cow A in experiment 1. However, because of the method of administration, considerable amounts of C11:0 may have passed out into the feces, but they were not examined. Higher amounts of C13:0 and C15:0 were observed in the milk but the magnitude of these increases was much smaller than with C11:0. Feeding of triundecanoin to rats led to odd-numbered carbon fatty acids longer than C11:0 in their tissues (7, 8). Chain elongation also occurred following intravenous infusion of tripelargonin in lactating cows (5). In compensation for the increases in odd-numbered carbon acids in the present experiment, the amounts of C16:0, C18:0, and C18:1 decreased both relatively and in absolute amounts produced.

TABLE IV

Feed Intake and Milk Production and Composition (Experiment 3).

Observation	Control[a]	Protected triundecanoin[b]
Feed intake (kg/day)	15.7	14.8
Milk production (kg/day)	9.2	10.0
Fat (%)	4.3	4.0**
Fat (kg/day)	0.39	0.39
Protein (%)	3.8	3.6
Protein (kg/day)	0.34	0.36
Solids-not-fat (%)	9.0	8.9
Solids-not-fat (kg/day)	0.83	0.88
Cholesterol (mg/100ml)	16.4	16.0

[a]Results for control periods before and immediately after the test treatment were included together.

[b]** = difference between control and treatment group significant at 1% level; no asterisk indicates no statistically significant difference.

To avoid the problems attendant upon the abomasal infusion, protected triundecanoin was fed in experiment 3. Feed intake was not lowered significantly (Table IV), nor was milk yield significantly affected. Fat percentage declined slightly but there was no difference in fat yield. Neither protein, nor SNF, nor milk cholesterol was significantly affected. Most of the even-numbered carbon fatty acids from C14:0 and higher showed a significant reduction in the relative amounts present in milk fat (Table V). The yields of these acids also declined, not always significantly, but enough to indicate that, as a group, the decrease to compensate for the icreased amounts of the odd-numbered carbon fatty acids was fairly general.

With protected triundecanoin, C11:0 in the milk rose to an average of 7.13% for the experimental period, far exceeding that with the unprotected triundecanoin. The highest levels attained by each of the two cows were 7.1 and 10.6%. These were far higher than those reached in experiment 1, even though the amounts of triundecanoin offered to the cows in experiment 3 were only ca. one-half

TABLE V

Relative Composition and Yields of Fatty Acids of Milk Fat (Experiment 3)

Fatty acid	Relative composition (wt %) Control[a]	Relative composition (wt %) Triundecanoin[b]	Amounts produced (g/day) Control[a]	Amounts produced (g/day) Triundecanoin[b]
C 4:0	3.6	3.8	14.0	14.2
C 6:0	1.7	1.7	6.5	6.6
C 8:0	1.2	1.2	4.7	4.5
C10:0	3.2	3.2	12.5	12.2
C12:0	4.0	4.3	15.8	16.5
C14:0	12.1	11.6*	47.5	44.3
C14:1	1.6	1.1**	6.6	4.3*
C16:0	30.6	26.9**	119.5	102.6
C16:1	2.8	2.2**	10.8	8.4*
C18:0	8.9	8.6	34.5	32.7
C18:1	20.2	17.2**	79.5	65.6
C18:2	2.5	2.7	9.6	10.3
C18:3	1.4	1.1**	5.4	4.3*
C 9:0	0.02	0.03**	0.07	0.11**
C11:0	0.6	7.1**	2.1	27.2**
C13:0	0.3	0.6**	1.1	2.4**
C15:0	1.8	3.0**	7.0	11.3**
C17:0	1.1	1.2	4.4	4.5

[a]Results for control periods before and immediately after the test treatment were included together.

[b]* = difference between control and treatment group significant at 5% level; ** = difference significant at 1% level; no asterisk indicates no statistically significant difference.

as much as those offered and even less than those consumed during experiment 1. C13:0 increased an average of 2.7 x, and C17:0 went up to 1.2 x their preexperimental control values. A small increase also was noted in the rather insignificant amount of C9:0 present. However, the amount of this latter fatty acid quickly returned to normal after feeding ceased. The increase, though small, was unexpected and perhaps idicated that some of the triundecanoin was being broken down to the propionic acid level and, as such, represented a larger amount available to the mammary gland for synthesis into higher odd-numbered carbon acids. Most of these fatty acids began to rise in the milk by the second day after feeding began, but C17:0 showed no increase until the third day and did not attain its maximum amount until the day after feeding ceased. These acids persisted in the milk in above normal levels for several days in continually diminishing amounts. All had returned to normal by the fifth day except for C11:0 which still remained slightly higher than usual. The actual amounts of fatty acids produced followed a similar pattern. Transfer of C11:0 from feed to milk amounted to 6.2 and 4.3% for the two cows, both well above the efficiency of transfer in experiments 1 and 2.

There would seem little doubt from the results of experiment 3 that the unprotected triundecanoin fed in experiment 1 was subject to considerable degradation in the rumen. Moreover, the failure of protected triundecanoin to have a significant effect upon feed intake, together with the previously discussed differences between the two cows in experiment 1, make it seem likely that the triundecanoin refusal noted with the unprotected material was due, not to an undesirable taste of the odd-numbered carbon triglyceride, but rather to an unfavorable metabolic reaction brought about by an inhibiting substance formed in the rumen. This unfavorable reaction in the rumen may be related to the bactericidal action of some of the odd-numbered carbon fatty acids, such as undecylenic acid (21).

REFERENCES

1. Garton, G.A., in "Digestive Physiology and Nutrition of the Ruminant," Edited by D. Lewis, Butterworths, London, England, 1961. p. 140.
2. Rook, J.A.F., Proc. Nutr. Soc. 18:117 (1959).
3. James, A.T., G. Peeters, and M. Lauryssens, Biochem. J. 64:726 (1956).
4. Patton, S., R.D. McCarthy, L. Evans, R.G. Jensen, and G.W. Gander, J. Dairy Sci. 45:248 (1962).
5. Storry, J.E., B. Tuckley, and A.J. Hall, Brit. J. Nutr. 23:157 (1969).
6. Zurier, R.B., R.G. Campbell, S.A. Hashim, and T.B. Van Itallie, Amer. J. Physiol. 212:291 (1967).
7. Campbell, R.G., and S.A. Hashim, Ibid. 217:1614 (1969).
8. Van Itallie, T.B., and A.K. Khachadurian, Science 165:811 (1969).
9. Bitman, J., L.P. Dryden, H.K. Goering, T.R. Wrenn, R.A. Yoncoskie, and L.F. Edmondson, JAOCS 50:93 (1973).
10. Storry, J.E., and D. Millard, J. Sci. Food Agr. 16:417 (1965).
11. Christopherson, S.W., and R.L. Glass, J. Dairy Sci. 52:1289 (1969).
12. Sobel, A.E., and A.M. Mayer, J. Biol. Chem. 1957:255 (1945).
13. Van Handel, E., Clin Chem. 7:249 (1961).
14. Annison, E.F., Aust. J. Agr. Res. 11:58 (1960).
15. Riddett, W., I.L. Campbell, F.H. McDowall, and G.A. Cox, N.Z. J. Sci. Technol. 23:80A (1941).
16. Allen, N.N., and J.B. Fitch, J. Dairy Sci. 24:516 (1941).
17. Van Soest, P.J., Ibid. 46:204 (1963).
18. Lough, A.K., in "Physiology of Digestion and Metabolism in the Ruminant," Edited by A.T. Phillipson, Oriel Press, Newcastle upon Tyne, England, 1969, p. 519.
19. Dawson, R.M.C., and P. Kemp, in "Physiology of Digestion and Metabolism in the Ruminant," Edited by A.T. Phillipson, Oriel Press, Newcastle upon Tyne, England, 1969, p. 504.
20. Storry, J.E., J.A.F. Rook, and A.J. Hall, Brit. J. Nutr. 21:425 (1967).
21. Stecher, P.G., M.K. Finkel, O.H. Siegmund, and B.M. Szafranski, "The Merck Index of Chemicals and Drugs," Seventh Edition, Merck and Co., Rahway, N.J., 1960, p. 1079.

[Received November 15, 1973]