EFFECT OF SKIM-MILK FEEDING ON THE FLOW AND COMPOSITION OF THORACIC DUCT AND INTESTINAL LYMPH IN YOUNG CALVES

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Summary

The effect of feeding skim milk on the flow and composition of lymph from thoracic and intestinal ducts was studied in young calves by means of lymphatico–venous shunts. The changes observed were similar for both thoracic duct and intestinal lymph. Skim-milk feeding significantly reduced lymph flow but had no effect on total protein concentration in lymph and no effect on lymph: plasma ratios for total protein concentration. It is suggested that the reduction in lymph flow observed when calves are fed skim milk is due to a decreased blood flow through the intestinal capillaries.

Skim-milk feeding substantially reduced the concentrations of all the lipid fractions in both lymph and plasma. Total lipid and triglyceride concentrations were much higher in lymph than in plasma and it was evident that some of the lipid in the lymph was derived endogenously.

I. INTRODUCTION

In young calves fed whole milk, the average flow of thoracic duct and intestinal lymph was 9·7 and 8·3 ml/kg/hr respectively (Shannon and Lascelles 1968). These values are considerably higher than those reported in other species of animals (Yoffey and Courtice 1956). It was suggested (Shannon and Lascelles 1967) that the continuous absorption of large amounts of lipid by the young calf was important in stimulating lymph flow. This suggestion is in conformity with the earlier observation of Simmonds (1955) that lipid absorption in rats was associated with an increase in the flow of thoracic duct lymph.

The aim of the present investigation has been to evaluate the influence of lipid on lymph formation in the young calf (7–21 days old) by studying the effects of feeding whole and skim milk on the flow and composition of thoracic duct and intestinal lymph.

II. MATERIALS AND METHODS

(*a*) *Animals*

Lymphatico–venous shunts were established in four Friesian bull calves. Details of the animals and the type of operation carried out on each are given in the following tabulation:

Calf number	1	2	3	4
Body weight (kg)	39	41	30	36
Lymphatic duct cannulated	Thoracic	Thoracic	Intestinal	Intestinal
Duration of lymph flow (days)	15	21	17	17

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The operations were carried out in the first week of life. The calves were taken from their dams at 1 day of age and taught to drink from a bucket. During the few days prior to the operation they were fed 2·27 litres (0·5 gal) of whole milk twice daily.

(*b*) *Surgical Techniques*

The surgical techniques used for the establishment of lymphatico–venous shunts between the thoracic duct and the left common jugular vein, and the intestinal duct and the left common jugular vein, have been described previously (Shannon and Lascelles 1967, 1968).

(*c*) *Management and Feeding*

Post-operative care and collection of samples were similar to that described by Shannon and Lascelles (1967). The calves were initially fed 2·27 litres (0·5 gal) of whole milk twice daily at 12-hr intervals, beginning on the first or second day after the operation. Two to three days were allowed on this feeding regime before the calves were sampled at hourly intervals for a period of 12 hr. The calves were then fed 2·27 litres (0·5 gal) of skim milk twice daily at 12-hr intervals, and after 2–3 days on this regime samples were again collected at hourly intervals for a period of 12 hr. At the completion of the skim-milk experiment, the calves were immediately returned to the original whole-milk feeding regime and after a further 2–3 days samples were collected at hourly intervals for a period of 12 hr.

(*d*) *Analytical Techniques*

The determination of total esterified fatty acid, phospholipid, and free fatty acid in lymph and plasma was carried out as described by Shannon and Lascelles (1967). The extraction of lipid in lymph and plasma samples with chloroform–methanol (2:1, v/v), and the analysis for triglyceride, esterified cholesterol, and free cholesterol by thin-layer chromatography, was carried out as described by Hartmann and Lascelles (1965). Total protein and albumin were estimated in plasma and lymph samples according to the procedure described by Gornall, Bardawill, and David (1949), as modified for determinations on chyle by Shannon and Lascelles (1968). Samples of whole milk fed to the calves were analysed for total fat by the Babcock method as described by Davis and MacDonald (1953). Skim-milk samples were analysed for total fat by extraction with chloroform–methanol (2:1, v/v).

III. Results

Comparisons of the average lymph flow, concentration and output of total protein, albumin:globulin ratio, and output of total esterified fatty acid, phospholipid, and free fatty acid in thoracic duct and intestinal lymph when the calves were alternately fed whole milk, skim milk, and whole milk, are presented in Table 1. It is clear from the results that the changes were similar for both thoracic duct and intestinal lymph. It may be seen that skim-milk feeding markedly reduced the output of total esterified fatty acid, phospholipid, and free fatty acid in thoracic duct and intestinal lymph. These changes were reversed when the calves were returned to whole milk. The average amount of total lipid ingested over a 12-hr period by the calves was 99·6, 3·7, and 110·2 g for whole-milk, skim-milk, and whole-milk feeding respectively.

A summary of the analysis of variance of the results for lymph flow and protein concentration from the four calves is presented in Table 2. The results analysed were derived from samples collected at hourly intervals over a 12-hr period for each of the three treatments. Thus, the results for the one period of skim-milk feeding have been compared with the two periods of whole-milk feeding. It may

TABLE 1

LYMPH FLOW, TOTAL PROTEIN CONCENTRATION AND OUTPUT, ALBUMIN:GLOBULIN RATIO, AND THE OUTPUT OF TOTAL ESTERIFIED FATTY ACID (T.E.F.A.) PHOSPHOLIPID, AND FREE FATTY ACID (F.F.A.) IN THORACIC DUCT LYMPH FROM CALVES 1 AND 2 AND INTESTINAL LYMPH FROM CALVES 3 AND 4

Values presented are means ± standard errors derived from 26 samples collected in each of three 12-hr periods when the calves were being fed whole milk, skim milk, and whole milk respectively

	Whole-milk Feeding		Skim-milk Feeding		Whole-milk Feeding	
	Thoracic Duct Lymph	Intestinal Lymph	Thoracic Duct Lymph	Intestinal Lymph	Thoracic Duct Lymph	Intestinal Lymph
Lymph flow (ml/hr)	430·6±25·7	363·8±22·2	276·1±18·0	175·1±22·8	365·2±11·1	287·3±20·1
Total protein concentration (g/100 ml)	3·64±0·14	3·84±0·06	3·69±0·18	4·01±0·05	3·68±0·15	4·21±0·09
Total protein output (g/hr)	15·62±1·19	13·64±0·68	10·21±0·85	6·99±0·27	13·76±1·03	11·81±0·67
Albumin: globulin ratio	1·05±0·05	0·86±0·02	1·14±0·05	0·94±0·02	1·25±0·06	0·91±0·03
T.E.F.A. output (g/hr)	4·598±0·462	6·915±0·506	0·448±0·063	0·448±0·039	6·552±0·573	5·285±0·418
Phospholipid output (g/hr)	0·589±0·047	0·928±0·049	0·187±0·015	0·142±0·011	0·772±0·063	0·640±0·054
F.F.A. output (g/hr)	0·087±0·008	0·062±0·004	0·015±0·002	0·014±0·001	0·072±0·006	0·050±0·004

be seen that there was a highly significant difference in the "between-treatments" effect for lymph flow. The significant ($P < 0{\cdot}001$) quadratic component, which accounted for 23% of the variation of the between-treatments term, was due to the reduction in lymph flow when the calves were fed skim milk (cf. Table 1). The significant ($P < 0{\cdot}05$) linear component of the between-treatments variation resulted from the somewhat lower lymph flow in the second period of whole-milk feeding compared with that observed in the first period. There was a significant variation ($P < 0{\cdot}01$) in lymph flow with time after feeding, which was common to all treatments and was similar to that reported previously for calves fed whole milk (Shannon and Lascelles 1967).

TABLE 2

SUMMARY OF THE ANALYSIS OF VARIANCE OF FLOW AND TOTAL PROTEIN CONCENTRATION IN THORACIC DUCT LYMPH (TWO CALVES) AND INTESTINAL LYMPH (TWO CALVES) COLLECTED AT HOURLY INTERVALS FOR 12 HR AFTER FEEDING, FOR TWO PERIODS OF WHOLE-MILK AND ONE PERIOD OF SKIM-MILK FEEDING

To calculate the variance ratio for the "treatments" source of variation, the mean square for "treatments × calves" has been used as the denominator, while the mean square for "treatments × calves × times" has been used as the denominator for the calculation of the variance ratios of the remaining sources of variation

Source of Variation	Degrees of Freedom	Mean Squares	
		Flow	Total Protein Concentration
Treatments	2	386,613***	0·5615
Linear	1	130,853*	1·1150
Quadratic	1	642,374***	0·0083
Times	12	20,889**	0·0873
Treatments × times	24	6,188	0·0729
Calves	3	128,003***	17·1546***
Treatments × calves	6	10,574	0·5778***
Times × calves	36	7,122	0·0585
Treatments × calves × times	72	7,137	0·0603

*$P < 0{\cdot}05$. ** $P < 0{\cdot}01$. *** $P < 0{\cdot}001$.

The differences between calves for lymph flow and protein concentration were primarily due to the fact that differences between thoracic duct and intestinal lymph were included in the "between-calves" term. The analysis also revealed that protein concentration in lymph of calves fed whole or skim milk was the same (cf. Table 1). Since the plasma protein concentration was found to be constant, it followed that lymph:plasma ratios for protein concentration were unaltered by skim-milk feeding. An analysis of variance of the results for albumin:globulin ratio also showed no significant difference between treatments.

TABLE 3

LIPID COMPOSITION OF PLASMA AND THORACIC DUCT LYMPH FROM CALVES 1 AND 2, AND PLASMA AND INTESTINAL LYMPH FROM CALVES 3 AND 4

Values (expressed as g/100 ml) are means ± standard errors for samples collected at various intervals over a 12-hr period when the calves were being fed skim milk. The number of samples is shown in parentheses

	Total Lipid	Triglyceride	Phospholipid	Free Fatty Acid	Esterified Cholesterol	Free Cholesterol
Calves 1 and 2						
Plasma (2)	0·183±0·007	0·009±0·003	0·078±0·006	0·004±0·001	0·076±0·000	0·012±0·002
Thoracic duct lymph (4)	0·261±0·062	0·121±0·049	0·070±0·011	0·004±0·001	0·037±0·006	0·008±0·001
Calves 3 and 4						
Plasma (2)	0·159±0·029	0·011±0·006	0·070±0·012	0·006±0·002	0·059±0·007	0·011±0·002
Intestinal lymph (4)	0·340±0·038	0·169±0·022	0·078±0·010	0·006±0·001	0·048±0·002	0·009±0·001

The composition of lipid in samples of thoracic duct and intestinal lymph collected at various intervals during the day following skim-milk feeding, is given in Table 3. For comparison, the lipid composition of plasma samples collected from the calves when they were being fed skim milk is included in this Table. Values for all the lipid fractions in both thoracic duct and intestinal lymph were substantially reduced by skim-milk feeding (cf. Shannon and Lascelles 1967, 1968). Plasma values were also substantially reduced by skim-milk feeding (cf. Shannon and Lascelles 1966).

IV. Discussion

It was considered that the experimental design eliminated the possibility of confounding the effects of age of the preparation with the effects of skim-milk *v.* whole-milk feeding. All the calves tolerated the changing dietary regime surprisingly well and remained in excellent health throughout the entire experimental period. The results unequivocally demonstrated that lymph flow and protein output in lymph were substantially higher when the calves were fed whole milk compared with that when skim milk was fed. It was also clear from the comparisons between calves with cannulated intestinal and thoracic lymph ducts that the differences in flow and composition observed with skim- and whole-milk feeding were due to changes in lymph formation in the intestines.

It may be seen that skim-milk feeding had no significant effect on the total protein concentration in either thoracic duct or intestinal lymph (Table 1). Since lymph:plasma ratios for total protein concentration were unaffected by skim-milk feeding it would appear that the decrease in lymph formation observed on skim-milk feeding was due to a reduction in capillary filtration area, or in other words, a decreased blood flow through the intestinal capillaries.

It is noteworthy that total lipid and triglyceride concentrations were much higher in lymph than in plasma following skim-milk feeding (Table 3). Since the output of total lipid in lymph over a 12-hr period following skim-milk feeding would have exceeded that available to the calf in the diet, it is evident that some of the lipid in the lymph was derived endogenously. In this connection, Balint, Spitzer, and Kyriakides (1963) and Baxter (1966) observed that endogenous lipid in lymph may be derived in part from bile. It was further suggested (Shrivastava, Redgrave, and Simmonds 1967) that fatty acid liberated from phospholipids of bile in the intestinal lumen contributed a major portion of the esterified fatty acid of fasting lymph in rats, and that lipoproteins passing from plasma into lymph provided much of the remainder. It is thought that a similar situation probably applies in calves fed skim milk.

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