The effect of heat treatment on the nutritive value of milk for the young calf

2.* The factor in a milk substitute associated with a high incidence of scouring and mortality

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It is now well established from our earlier experiments, based on the classical work of Smith & Little (1922), that survival of the newborn calf is associated with the ingestion of specific antibodies to *Escherichia coli* and that these antibodies are associated with the globulin fraction of colostrum (Aschaffenburg, Bartlett, Kon, Terry, Thompson, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Walker, Briggs, Cotchin & Lovell, 1949; Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs & Lovell, 1951; Briggs, 1951; Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thompson & Walker, 1951; Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Roy, Palmer & Shillam, 1956). In the absence of colostrum a fatal septicaemia may occur during the first few days of life, since colostrum containing specific antibodies apparently prevents invasion of the tissues. However, colostrum does not necessarily prevent multiplication of the organisms in the alimentary tract during early life; such multiplication is frequently associated with severe diarrhoea and dehydration and may eventually lead to death. This condition, commonly known as 'white scours', is undoubtedly the most important cause of calf mortality in Great Britain (Withers, 1953). Further, affected calves that recover often fail to gain weight normally until some time after they are weaned from a liquid diet.

Roy, Palmer, Shillam, Ingram & Wood (1955) demonstrated that the level of 'infection' in a calfhouse, as indicated by the incidence of scouring and mortality of colostrum-fed calves, increased with the length of time that the calfhouse had been occupied after a period of vacancy; it was suggested that this build-up of 'infection' resulted from the gradual dominance of certain 'pathogenic' strains of *E. coli* (Wood, 1955). Even so, it appeared that the build-up might be at least partly associated with the diet, for the studies of Roy *et al.* (1955) indicated that the rate of increase in the level of 'infection' tended to be lowest in calves given a diet of whole milk. The first experiment reported now was made to confirm our observation that a 'synthetic milk'

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used as the basal diet in our earlier studies predisposed colostrum-fed calves to scouring and mortality, once an 'infection' had built up in the calfhouse. The 'synthetic milk' was isocaloric with average whole milk and was of the following constant percentage composition: spray-dried skim milk 9·8, non-vitaminized margarine 2·0, glucose 2·5, water 85·7. In addition, 3500 i.u. vitamin A and 700 i.u. vitamin D were given to all the calves daily. The second experiment showed that the dried skim-milk fraction of this 'synthetic milk' was associated with the high mortality rate. Subsequent experiments made to determine the reason for this effect will be reported later. In addition, the effect of heat treatment of milk on the performance of the calf reared under conditions of low 'infection' has been studied. The effect of ultra-high-temperature (UHT) treatment and of pasteurization of milk on the performance of the calf has already been recorded (Shillam, Dawson & Roy, 1960).

METHODS

Plan of experiments

Both experiments were of randomized block design. Calves were allotted, as they were born, to treatment selected at random within each block. Expt 1 was done in the winter months of 1952–3 and consisted of three treatments in each of eight blocks; Expt 2 was done in the autumn and winter months of 1954 and consisted of six treatments in each of fifteen blocks. Shorthorn calves only were used in Expt 1, but in Expt 2 Shorthorns and Ayrshires were used, each block consisting of one breed of calf only.

Diets

Colostrum. Colostrum was obtained within 24 h of calving from the same farms that supplied the calves. The fat was first removed in an ordinary cream separator and the separated colostrum was stored in waxed cartons at $-25°$. Each calf was given as its first meal, 400 ml separated colostrum consisting of 100 ml from each of four different batches. Calves within each block received the same blend of colostrum, but there were differences in the blend between some of the blocks. The amount of colostrum was restricted to 400 ml as previous experiments had shown that this amount was usually adequate to protect against an *E. coli* septicaemia.

Basal diets. The composition of the diets in the various treatments is given in Table 1.

The whole milk used for the diets of calves in treatments 6, 11 and 12 was bulked milk from the Institute herd. For diets containing margarine, a milk of 20% fat content was prepared by homogenizing 2 lb of non-vitaminized margarine into a reconstituted mixture of 0·8 lb spray-dried skim milk in 7·2 lb water. The homogenizer used has been described previously (Roy, Shillam, Thompson & Dawson, 1961). Each 10 lb batch of the diets used in treatments 4 and 7 was prepared by reconstituting 0·9 lb skim-milk powder and 0·25 lb glucose with 7·85 lb water to which was added 1 lb of the milk of 20% fat content; for treatment 8, the glucose was omitted from this mixture and was replaced by an equivalent weight of water. These diets in which the basis was spray-dried skim-milk powder will be referred to subsequently as 'synthetic milks'. For treatments 5 and 10, the diets were prepared by adding 1 lb of the milk of 20% fat content to each 9 lb of bulked separated milk from the Institute herd. For treatment 9, 1 lb of the milk containing 20% fat and 0·25 lb glucose were added to each 8·75 lb of separated milk. The spray-dried skim milk was purchased in large batches to ensure a product of constant composition throughout each experiment.

Table 1. *Composition of the basal diets*

	Expt 1			Expt 2					
	Treatment no.								
Component	4	5	6	7	8	9	10	11	12
Non-vitaminized margarine (%)	2·0	2·0	—	2·0	2·0	2·0	2·0	—	—
Glucose (%)	2·5	—	—	2·5	—	2·5	—	—	—
Spray-dried skim milk (%)	9·8	0·8	—	9·8	9·8	0·8	0·8	—	—
Fresh separated milk (%)	—	90·0	—	—	—	87·5	90·0	—	—
Whole milk (%)	—	—	100	—	—	—	—	100	100
Water (%)	85·7	7·2	—	85·7	88·2	7·2	7·2	—	—
Vitamin A (i.u./calf daily)	3500	3500	—	3500	3500	3500	3500	3500	—
Vitamin D (i.u./calf daily)	700	700	—	700	700	700	700	700	—

Each calf that was given diets from which the butterfat had been removed received a supplement of vitamin A in the form of halibut-liver oil concentrate and synthetic vitamin D_3, dissolved in arachis oil. This supplement was also added to the diet of one group of calves given whole milk in Expt 2 (treatment 11). Treatment 11 was included to provide a valid comparison of the effect of the butterfat in whole milk with that of the margarine added to fresh separated milk (treatment 10).

Calves

The calves were collected within a few hours of birth from tubercle-free herds in the Shinfield area. Before they were placed on experiment, blood was drawn from the jugular vein of each calf and the globulin-turbidity test (Aschaffenburg, 1949) was applied to the serum to verify that the calf had not suckled its dam. The housing arrangement was similar to that of earlier experiments (Aschaffenburg, Bartlett, Kon, Terry *et al.* 1949). The calves were pail-fed three times daily for the first 10 days of life and thereafter twice daily at the rate of 1 lb diet/10 lb live weight until they were 3 weeks of age. When a calf that was given three feeds daily scoured, one feed was omitted and thereafter the diet was reduced to that calculated to maintain body-weight (Roy, Shillam, Hawkins & Lang, 1958). When the consistency of faeces became normal, the volume of milk was increased to the normal daily allowance. When a calf that was given two feeds daily scoured, a similar routine was adopted except that one feed was not omitted. The procedure was repeated when scouring recurred. For each calf, the daily consistency of the faeces was recorded subjectively. Dry-matter determinations of the faeces of calves in later experiments showed that the dry-matter content of 'scours' was < 10% compared with 14% for 'loose' faeces, 22–26% for normal faeces and > 28% for firm faeces (see Roy *et al.* 1961).

Rectal temperatures were recorded daily, those above 102·8 °F being regarded as high (Aschaffenburg, Bartlett, Kon, Roy, Sears, Thompson, Ingram, Lovell & Wood, 1953).

A record was made also of the time taken by each calf to pass its meconium completely. There is strong evidence (see Roy, 1956) that, in calves given a small amount of colostrum, a rapid rate of passage of meconium is associated with a high degree of intestinal disturbance.

The calves were weighed daily on a steel-yard type machine accurate to 0·25 lb, and the mean daily live-weight gain over the 3-week period was obtained by fitting a linear regression to the daily weights.

Analytical methods

The partition of nitrogen in a sample of the spray-dried skim milk used in Expt 2 was determined by the method of Aschaffenburg & Drewry (1959).

RESULTS

Expt 1

The results are given in Tables 2 and 3. All eight calves given the synthetic milk died, compared with three given the diet containing fresh separated milk and only one given whole milk. The χ^2 test, adjusted for continuity, showed the differences to be significant. Of the calves that died, eight showed at autopsy a localized intestinal infection with *E. coli*. Surviving calves scoured severely and weight gains were extremely subnormal.

Table 2. *Expt* 1. *Comparison of the performance (mean values with their standard errors) of calves given a synthetic milk, separated milk containing 2% fat, or whole milk*

	Treatment no. and diet			
	4 Synthetic milk	5 Separated milk containing 2% fat	6 Whole milk	Significance of difference between treatments
Calves				
No. used	8	8	8	—
No. died	8	3	1	4 > 5,* 4 > 6**
Age at death (days)	9 ± 2·1	7 ± 0·6	4†	—
Live-weight gain/day of surviving calves (lb)	—	−0·08 ± 0·15	−0·08 ± 0·13	—
No. of days on which surviving calves scoured	—	8 (range 6–14)	8 (range 5–11)	—
No. of days on which surviving calves had a high rectal temperature (> 102·8 °F)	—	0 (range 0–1)	1 (range 0–3)	—

* Significant at 0·01 < *P* < 0·05.
** Significant at 0·001 < *P* < 0·01.
† One calf only.

Table 3. *Expt* 1. *Number of calves that died, classified according to post-mortem findings*

Post-mortem finding	Treatment no. 4 Synthetic milk	5 Separated milk	6 Whole milk
E. coli septicaemia	1	—	1
E. coli septicaemia and polyserositis	—	1	—
E. coli localized intestinal infection	7	1	—
Unclassified intestinal infection	—	1	—

Expt 2

Mortality. The results are given in Table 4. Seventeen of the thirty calves given the synthetic milk either with or without glucose died, compared with only seven of thirty given the diets containing fresh separated milk and only nine of thirty given whole milk. The χ^2 test, adjusted for continuity, showed that the difference in mortality rate between calves given the synthetic milk and those given diets containing fresh separated milk was significant ($0.01 < P < 0.05$) but the difference in

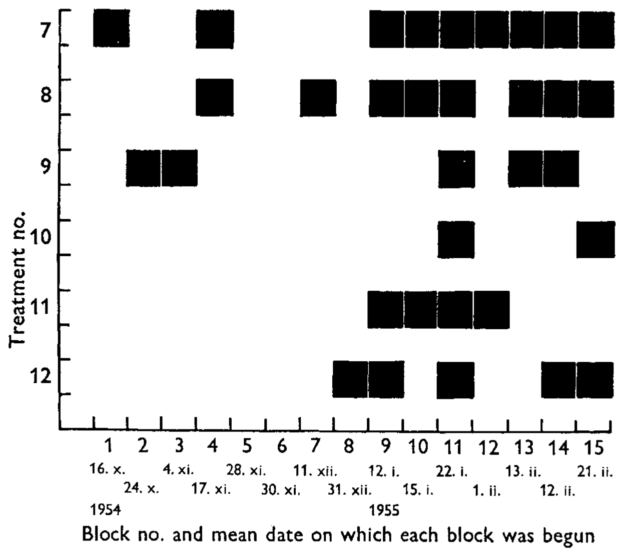

Fig. 1. Expt 2. Distribution of mortality on each treatment. 1, synthetic milk; 2, synthetic milk without glucose; 3, fresh separated milk plus glucose; 4, fresh separated milk; 5, whole milk plus supplementary vitamins A and D; 6, whole milk. Each square symbol represents one death.

mortality rate between calves given the synthetic milk and those given whole milk just lacked significance. Fourteen of the thirty calves given glucose either with the synthetic milk (treatment 7) or with the fresh separated milk (treatment 9) died compared with ten of the thirty given these basal diets without glucose (treatments 8 and 10); the effect of glucose was not significant. There was no significant difference in the mortality rates of calves given whole milk and of those given whole milk with supplementary vitamins A and D.

Table 4. *Expt 2. Comparison of the performance of calves (mean values with their standard errors) given the synthetic milk with and without glucose, fresh separated milk containing 2 % fat with and without glucose or whole milk with and without added vitamins*

	Treatment no. and diet						
	7 Synthetic milk	8 Synthetic milk without glucose	9 Separated milk containing 2 % fat plus glucose	10 Separated milk containing 2 % fat	11 Whole milk plus vitamins A and D	12 Whole milk	Significance of difference between treatments
Calves							
No. used	15	15	15	15	15	15	—
No. died	9	8	5	2	4	5	—
	17		7		9		7+8 > 9+10*†
Age at death (days)	6 ± 0·8	6 ± 1·0	7 ± 0·9	10 ± 0·5	8 ± 0·9	7 ± 0·2	—
	6 ± 0·6		8 ± 0·8		8 ± 0·4		—
Live-weight gain/day of surviving calves (lb)	0·52 ± 0·11	0·21 ± 0·10	0·22 ± 0·09	0·22 ± 0·08	0·26 ± 0·08	0·44 ± 0·09	—
Birth weight of surviving calves (lb)	80·1 ± 3·5	79·4 ± 3·3	76·5 ± 2·7	75·6 ± 2·4	82·4 ± 2·6	80·9 ± 2·7	—
Milk consumption of surviving calves (pints)	117·5 ± 6·3	111·1 ± 5·8	109·1 ± 4·9	109·9 ± 4·3	118·4 ± 4·6	124·0 ± 4·9	—
No. of days on which surviving calves scoured	5 (range 1–11)	5 (range 1–9)	5 (range 1–10)	5 (range 2–11)	5 (range 2–9)	4 (range 1–7)	—
No. of days on which surviving calves had a high rectal temperature (> 102·8 °F)	0 (range 0–1)	1 (range 0–2)	1 (range 0–4)	1 (range 0–5)	2 (range 0–7)	1 (range 0–3)	—
Adjusted live-weight gain/day of surviving calves (lb)‡	0·46 ± 0·07	0·27 ± 0·06	0·29 ± 0·05	0·24 ± 0·06	0·28 ± 0·05	0·31 ± 0·05	7 > 8*
Time between birth and complete passage of meconium (h)	33·9 ± 2·2	34·4 ± 1·7	37·8 ± 1·9	37·7 ± 1·9	38·1 ± 2·0	39·2 ± 1·8	11+12 > 7+8*†

* Significant at $0·01 < P < 0·05$.

† Two treatment nos. joined by a plus sign denote the mean value for those treatments.

‡ Adjusted for differences between treatment groups in mean birth weight, milk consumption and incidence of a high rectal temperature.

The distribution of the deaths on each treatment for each block of the experiment is shown in Fig. 1, from which it can be seen that the mortality rate increased with increasing time of occupation of the calfhouse. This increase in mortality was more marked among calves given the synthetic milk than among those given the diets containing fresh separated milk or whole milk. Thus in the last seven blocks of the experiment, thirteen of fourteen calves given the synthetic milk died, compared with five of fourteen given the fresh separated milk diet and eight of fourteen given whole milk.

There was some evidence that, of the calves that died, those given the basal diet of synthetic milk tended to succumb at an earlier age than those given the diets containing fresh separated milk or whole milk.

Autopsy. All thirty-three calves that died showed at autopsy a localized intestinal infection with *E. coli*. *Listeria monocytogenes* was isolated from the liver and mesenteric lymph nodes of two of these calves, both of which had received the synthetic milk.

Performance of surviving calves. There were no significant differences in the observed weight gains of the surviving calves on the six treatments as given in Table 4, although there was a tendency for the weight gains of calves given the synthetic milk to be greater than those of calves given the synthetic milk without glucose. After adjustment of the mean daily live-weight gains for differences between treatment groups in mean birth weight, total milk consumption and incidence of a high rectal temperature, the difference in weight gains between calves on treatment 7 (given glucose) and those on treatment 8 (not given glucose) was significant. A similar trend was apparent in the adjusted mean weight gain of those calves given the diets containing fresh separated milk with and without glucose. The difference in adjusted mean weight gain of surviving calves given the basal diets of dried skim milk and fresh separated milk containing glucose (treatments 7 and 9) and those given these basal diets with the glucose omitted (treatments 8 and 10) just lacked significance. The relevant partial regression coefficients with their standard errors are given below; the adjusted mean values are given in Table 4.

	General mean	Partial regression coefficient with its standard error
Live-weight gain/day (lb)	0·294	—
Birth weight (lb)	79·02	$-0·0151 \pm 0·0036$***
Total milk consumption (pints)	114·80	$+0·0177 \pm 0·0020$***
Incidence of a high rectal temperature (values transformed thus $\sqrt{(x+\frac{1}{2})}$)	1·169	$-0·104 \pm 0·041$*

* Significant at $0·01 < P < 0·05$. *** Significant at $P < 0·001$.

Calves given whole milk or the fresh separated milk diets passed their meconium at a slower rate than those given the synthetic milk; the difference in rate of passage of meconium between calves given the synthetic milk and those given whole milk was significant.

Partition of nitrogen in the dried skim milk. The partition of nitrogen in a sample of the dried skim milk used in Expt 2, reconstituted at the rate of 1 lb milk powder/9 lb water, is given in Table 5. For comparison, values for the partition of the nitrogen fractions, calculated for normal cow's milk containing the same amount of total N as the reconstituted skim milk (R. Aschaffenburg, 1955, private communication), are also given. It can be seen that the dried milk contained only about 50% of the non-casein proteins present in normal milk. The albumin fraction was particularly low; its content was about 40% of that in normal milk. Values for proteose-peptone N and globulin N were determined, but since the globulin N value was obtained by difference from two rather large values, a composite figure for proteose-peptone and globulin N is given in Table 5.

Table 5. *Nitrogen partition (mg N/100 g milk) of the dried skim milk used in Expt 2 and of normal milk*

	Reconstituted dried skim milk*	Normal milk†
Total N	512	512
Casein N	437‡	390
Non-casein N	75	122
Non-casein protein N	47	94
Total albumin N	27	70
Proteose-peptone + globulin N	20	24
Non-protein N	28	28

* Reconstituted at the rate of 1 lb milk powder/9 lb water.
† Values of N fractions calculated for normal milk containing 512 mg total N/100 g milk (R. Aschaffenburg, 1955, private communication).
‡ Includes denatured non-casein protein N.

DISCUSSION

The results of these experiments show quite clearly that in calves given a similar degree of immunity from colostrum, the incidence of scouring and mortality associated mainly with an *E. coli* localized intestinal infection is higher when the calves are subsequently given the synthetic milk than when they are given whole milk. The difference between the mortality rate of calves given similar diets in the two experiments was probably due to the different levels of 'infection' in the calfhouse at the time of each experiment. The first experiment, in which all calves given the synthetic milk died, began after the calfhouse had been in use for 3 months after a period of vacancy; the 'infection' level was probably high. When Expt 2 began, twenty calves only, none of which had died, had passed through the calfhouse after a period of vacancy of 5 months. Although fourteen of the first eighteen calves given the synthetic milk (treatments 7 and 8) in Expt 2 survived the experimental period, the 'infection' appears to have built up to such a level by the end of the ninth block that one only of the last fourteen calves given these diets survived. At this time an unusually large number of calves given the diets containing fresh separated milk or whole milk also died. However, of the calves that died, those given the latter diets tended to succumb at a later age, and thus seemed to offer a greater resistance, than those given the synthetic milk. Further evidence that the digestive upsets were more severe among calves given the synthetic milk is provided by the fact that these calves passed their meconium at a significantly faster rate than those given whole milk.

The results of Expt 2 show also that the dried skim milk is the component of the synthetic milk responsible for this high mortality rate. The possibility of any other constituent being implicated can be eliminated. Thus, as the mortality rate of calves given fresh separated milk containing 2 % margarine and of those given whole milk was similar in both experiments, it was unlikely that the margarine in the synthetic milk was detrimental. It was considered possible that the addition of glucose at the level of 2·5 % in the synthetic milk might be responsible for the high mortality rate since Rojas, Schweigert & Rupel (1948) and Blaxter & Wood (1953) have shown that, owing to an increase in bacterial proliferation in the intestine, diarrhoea and unthriftiness may often be associated with intakes of over 200–250 g sugar daily. However, in Expt 2 a calf of, say, 80 lb body-weight given daily $6\frac{1}{2}$ pints of the basal diet containing added glucose ingested about 90 g glucose daily in addition to the 180–190 g lactose in the basal diet, yet clearly there was no adverse effect on mortality rate or on the incidence of scouring in surviving calves. In fact, the inclusion of glucose in the diets increased weight gains slightly, a finding that was no doubt due to the increase in calorific value.

The dried skim milk was manufactured by the Gray–Jensen process in which the liquid milk was first precondensed by heating at 74° for periods up to an hour (the average holding time was about 30 min), and then sprayed ('atomized') into heated air circulating through a drying chamber. Full details of the operation of the plant are given by Hunziker (1949). From the commercial aspect, preheating of milk before drying is desirable for it increases the capacity of the drier and less steam is needed at high temperatures (Crossley, 1945–6). Further, it enables heat transfer to occur in the shortest possible time in a drying chamber of reasonable dimensions. Preheating also controls the bacterial content of the powder (Mattick, Hiscox & Crossley, 1945–6) and extends the storage life of whole-milk powder, mainly by the production of sulphydryl compounds which act as antioxidants, and also by the destruction of oxidizing enzymes (Mattick, Hiscox, Crossley, Lea, Findlay, Smith, Thompson, Kon & Egdell, 1945–6; White, Smith & Lea, 1947–8).

The effect of heat on the denaturation of the whey protein fraction of milk has been the subject of much study since the early work of Rowland (1933–4, 1937) who showed that heating milk at 75° for 15 min denatured 85 % of the albumin and globulin N and that denaturation was more rapid and complete at temperatures greater than 75°. Rowland (1933–4, 1937) demonstrated also that heat denaturation of whey proteins is affected by the duration of heating as well as by the temperature employed. This time–temperature relationship was studied in more detail by Larson & Rolleri (1955), who showed that heating milk at a temperature of only 56° for 30 min denatured 2–3 % of the whey proteins, and by Harland, Coulter & Jenness (1952), who demonstrated that exposure at 74° for only 2 min was sufficient to denature more than 10 % of the whey proteins in milk.

Henry, Kon, Lea & White (1947–8) found that lactalbumin N and lactoglobulin N comprised 5·5 % of the total N in a commercial dried skim milk produced in a Gray–Jensen plant compared with an average value of 12·5 % for liquid milk (Rowland, 1938). Similarly, Harland *et al.* (1952) and Larson & Rolleri (1955) found that 40–50 %

of the whey proteins of skim milk were denatured by heating at 74° for 30 min. The apparent denaturation of 50% of the whey proteins in the skim milk used by us, which had been subjected to a similar time–temperature relationship during the drying process, is in good agreement with these reported values. Henry *et al.* (1947–8) presumed that their observed changes in N distribution were largely caused by the preheating treatment of the liquid milk. This assumption is probably correct since Harland *et al.* (1952) found that most of the heat denaturation of the whey proteins must have occurred in the evaporator, since the time and temperature to which the milk particles were exposed during the spray-drying operation itself were not sufficient to cause measurable denaturation; Roy (1956) showed that with colostrum that was not preheated, less than 5% of the whey proteins were denatured during the spray-drying process.

That the spray-drying process may affect the nutritive value of milk for the rat was shown by Nevens & Shaw (1933), who found that the apparent digestibility of the proteins of whole-milk powder was appreciably less than that of the proteins of liquid whole milk. Fairbanks & Mitchell (1935) found that preheating skim milk at 63° for an unspecified time did not reduce the digestibility for the rat but that it lowered the biological value of the proteins by about 8%. On the other hand, Henry, Houston, Kon & Osborne (1939) found that the biological value of the proteins of spray-dried milk was unaffected by preheating at 71° (160 °F) for 1–4 sec, and Henry & Kon (1947–8) found only small non-significant differences in true digestibility and biological value when this preheating temperature was raised to 88° (190 °F) for 5 min.

The importance of colostral whey proteins was stressed by Aschaffenburg *et al.* (1951) who demonstrated that only very small amounts were necessary to protect the calf against an *E. coli* septicaemia. From the findings reported here it appears probable that, in calves reared under conditions of high 'infection', the undenatured whey protein fraction of milk is essential to prevent the onset of a localized intestinal infection with *E. coli*. It is perhaps relevant to compare these results with our finding reported earlier that whole milk in which 72% of the whey proteins had been denatured by UHT treatment significantly reduced the growth rate of calves reared under conditions of low 'infection' (Shillam *et al.* 1960).

SUMMARY

1. In two experiments with 114 colostrum-fed calves, the mortality rate was significantly greater with a synthetic milk diet than with a diet containing fresh separated milk or whole milk.

2. Spray-dried skim milk was the component of the synthetic milk that was associated with the high mortality rate.

3. About 50% of the whey proteins of the dried skim milk were apparently denatured during the drying process.

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