

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

5*. A comparison of spray-dried skim milks prepared with different preheating treatments and roller-dried skim milk, and the effect of chlortetracycline supplementation of the spray-dried skim milks

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Earlier experiments in this series have been made under conditions of moderate to high 'infection' in the calfhouse and with calves that had been given small amounts of colostrum, sufficient only to protect against death from an *Escherichia coli* septicæmia (Shillam, Roy & Ingram, 1962*a-c*). Under these conditions, diets containing the more severely heated milks predisposed calves to scour, which in many instances eventually led to death associated with a localized intestinal infection with *E. coli*, the usual post-mortem finding in those colostrum-fed calves that die. In general, the weight gains of surviving calves given these milks have been subnormal, but this poor gain did not appear to be due entirely to the high incidence of scouring. In the statistical analysis of these experiments, adjustment of weight gains was normally made for the effects of between-treatment differences in the incidence of scouring, but no allowance was possible for any detrimental effect on utilization of the diet that may have resulted from subclinical digestive disturbances. Further, it has not been possible to allow for any differences in severity of scouring or for any difference in effect between continuous and intermittent periods of scouring. The experiments described now were made to compare the nutritive value of the spray-dried skim milks that had been given different preheating treatments before drying (Shillam *et al.* 1962*b*). In contrast to the earlier experiment, the calves were given initially an amount of colostrum that was more in keeping with that given in practice and were reared under conditions in which, it was hoped, little or no scouring would occur. In an endeavour to overcome any possible subclinical digestive disorder, chlortetracycline was added to the diets in one of the experiments; the beneficial action of chlortetracycline in the prevention of digestive disorders is well known (see reviews by Lassiter, 1955; Roy, Shillam, Palmer & Ingram, 1955). In the comparison a diet containing skim milk dried by the roller process was included; roller-dried skim milk is subjected to a comparatively severe heat treatment during manufacture, and is widely used by the animal feeding-stuffs industry (Commonwealth Economic Committee: Intelligence Branch, 1960).

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METHODS

Experimental design and duration of trial

Expts 1 and 2. Expt 1 was done in the early spring months of 1958 and Expt 2 in the early spring months of 1959; they were of randomized block design with twelve blocks per treatment:

	Treatment no.	Colostrum	Milk	Chlortetracycline
Expt 1	22	6 pints whole colostrum	Milk A (spray-dried skim milk, 'severe' heat treatment, containing 2 % fat)	—
	23		Milk B (spray-dried skim milk, 'mild' heat treatment, containing 2 % fat)	—
Expt 2	24		Milk A (as above)	None
	25		Milk A (as above)	125 mg daily
	26		Milk B (as above)	None
	27		Milk B (as above)	125 mg daily
	28	Roller-dried skim milk containing 2 % fat	None	

The calves were on experiment for the first 3 weeks of life.

Expt 3. Digestibility and balance trials with four pairs of calves were made. The calves of each pair were born on the same day or within a day or two of each other so that both were exposed to the same environmental conditions. Each calf was given 6 pints of whole colostrum; one of each pair was then given milk A and the other milk B. The duration of the metabolism trial was 7 days, beginning at the afternoon feed on the 3rd day of life.

Calves

Ayrshire and Shorthorn bull calves were used; management was as described earlier (Shillam *et al.* 1962*c*). In Expt 3, the calves were weighed immediately before the afternoon feeds on the 3rd and 10th days of life.

Diets

Colostrum. Each calf was given 6 pints of whole colostrum within the first 36 h of birth, consisting of 1 pint from each of six different batches.

Milk. The spray-dried skim milks were prepared by the processes described earlier (Shillam *et al.* 1962*a, b*); milk A was initially preheated at a temperature of 74° for about 30 min ('severe' heat treatment) and milk B at 77° for 15 sec ('mild' heat treatment). The roller-dried skim milk was prepared in a commercial plant; the liquid milk was preheated to 110° in a Stork heater and then precondensed to 25% total solids in a triple effect APV plate evaporator. The milk was then dried on rollers operating at a steam pressure of 50 lb/in²; the approximate surface temperature of the rollers was 116°.

The skim milks were reconstituted at the rate of 1 lb milk powder/9 lb water, and 1 lb of a milk of 20% fat content (Shillam *et al.* 1962*a*) was added to each 9 lb of milk.

After the colostrum-feeding period, the diets were given at a rate that had been found adequate for a growth rate of 1 lb/day with whole milk of 3.3% fat content (Roy, Shillam, Hawkins & Lang, 1958). The usual daily supplement of 3500 i.u. vitamin A in the form of halibut-liver oil concentrate and 700 i.u. synthetic vitamin D₃, dissolved in arachis oil, was given to each calf.

Chlortetracycline. Chlortetracycline hydrochloride (Cyanamid of Great Britain Ltd), 125 mg, was given at the evening feed to each calf on treatments 25 and 27.

Collection of faeces and urine

The calves in Expt 3 were placed in individual metabolism crates on the 3rd day of life. Faeces were collected in 18 × 20 in. Polythene bags of 1–2 × 10⁻³ in. wall thickness which were prepared in a manner similar to that described by Noller, Ward, McGilliard, Huffman & Duncan (1956). Urine was collected in 2.5 l. bottles containing 25 ml glacial acetic acid.

Analytical methods

Milk. The proximate compositions of the dried skim milks used in Expts 1 and 2, after reconstitution at the rate of 1 lb milk powder/9 lb water but without the addition of the 20% fat milk, and of the diets given to the calves in Expt 3 were determined by the methods used by Rowland, Roy, Sears & Thompson (1953). The partition of nitrogen in the roller-dried milk was determined by the method of Aschaffenburg & Drewry (1959).

Faeces and urine. N was determined in urine and in acidified faeces by the Kjeldahl method. The remainder of the faeces was dried to constant weight at 100° and the dry-matter content calculated.

RESULTS

Expts 1 and 2

The results are given in Table 1. All calves survived the 3-week experimental period. In both experiments the mean incidence of scouring was low and did not differ significantly between treatments. There was, however, a considerable variation within treatments in the incidence of scouring which was not reduced by supplementation with 125 mg chlortetracycline/day. It is not surprising therefore that the antibiotic had no significant effect on weight gains. There were no differences between treatment means in the time interval between birth and complete passage of meconium. In Expt 2, calves given the diet containing roller-dried milk had on average a significantly higher incidence of a high rectal temperature than calves on the other treatments.

The outstanding feature of both experiments was the large difference between the milk diets in the live-weight gain of the calves. In Expt 1 the observed mean daily weight gain of calves given milk A (spray-dried, 'severe' heat treatment) was significantly less (0.001 < *P* < 0.01) than that of calves given milk B (spray-dried, 'mild' heat treatment). This difference was confirmed in Expt 2 which showed also that the mean weight gain of calves given the roller-dried skim milk was significantly less

Table 1. Expts 1 and 2. Comparison of the performance (mean values with their standard errors or ranges) of calves given diets containing spray-dried skim milk prepared with different preheating treatments or roller-dried skim milk, and effect of chlortetracycline supplementation of the spray-dried skim milks

	Expt 1				Expt 2				Significance of difference between treatments
	Treatment no. and diet				Treatment no. and diet				
	22	23	24	25	26	27	28		
No. of calves used	12	12	12	12	12	12	12	—	
Live-weight gain/day (lb)	0.38 ± 0.04	0.60 ± 0.04	0.42 ± 0.04	0.48 ± 0.04	0.72 ± 0.04	0.62 ± 0.04	0.46 ± 0.04	22 < 23**, 24 < 26***, 24 < 27***, 25 < 26***, 25 < 27*, 28 < 26***, 28 < 27**, 24 + 25 < 26 + 27***†	
No. of days on which calves scoured	2 (range 0-4)	2 (range 0-4)	2 (range 0-6)	1 (range 0-6)	1 (range 0-6)	2 (range 0-5)	1 (range 0-3)	—	
No. of days on which calves had a high rectal temperature (> 102.8° F)	1 (range 0-5)	2 (range 0-8)	1 (range 0-7)	1 (range 0-4)	1 (range 0-4)	2 (range 0-6)	2 (range 1-5)	24 + 25 + 26 + 27 < 28*†	
Birth weight (lb)	77.9 ± 2.4	74.7 ± 2.4	71.2 ± 3.0	75.5 ± 3.0	78.0 ± 3.0	75.8 ± 3.0	74.4 ± 3.0	—	
Milk consumption (pints)	149.1 ± 2.5	145.4 ± 2.5	142.7 ± 4.0	148.9 ± 4.0	153.7 ± 4.0	152.2 ± 4.0	147.7 ± 4.0	—	
Time between birth and complete passage of meconium (h)	45.1 ± 3.8	49.0 ± 4.1	41.3 ± 4.6	39.9 ± 4.2	43.2 ± 4.0	41.2 ± 4.6	41.7 ± 4.2	—	
Adjusted live-weight gain/day (lb)†	0.39 ± 0.03	0.59 ± 0.03	0.45 ± 0.03	0.48 ± 0.03	0.68 ± 0.03	0.60 ± 0.03	0.49 ± 0.03	22 < 23**, 24 < 26***, 24 < 27**, 25 < 26***, 25 < 27*, 28 < 26***, 28 < 27**, 24 + 25 < 26 + 27***†	

* Significant at 0.01 < P < 0.05.

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

‡ Adjusted for differences between treatment groups in mean birth weight (Expt 1), and mean birth weight, milk consumption and incidence of a high rectal temperature (Expt 2).

($P < 0.001$) than that of calves given milk B. Multiple covariance analyses of live-weight gain/day on the variables birth weight, milk consumption, incidence of scouring and incidence of a high rectal temperature were made. In Expt 1, only the partial regression coefficient of live-weight gain/day on birth weight was significant. Treatment means were therefore adjusted for differences between treatment groups in mean birth weight by means of the simple regression coefficient of live-weight gain/day on birth weight given below. In Expt 2, mean daily weight gains were adjusted for differences between treatment groups in mean birth weight, milk consumption and incidence of a high rectal temperature by means of their partial regression coefficients.

	General mean	Regression coefficient with its standard error
Expt 1 Live-weight gain/day (lb)	0.489	—
Birth weight (lb)	76.29	-0.0103 ± 0.0038†*
Expt 2 Live-weight gain/day (lb)	0.538	—
Birth weight (lb)	74.97	-0.0103 ± 0.0037‡***
Milk consumption (pints)	149.03	+0.0116 ± 0.0028‡***
No. of days on which calves had a high rectal temperature (values transformed $\sqrt{(x + \frac{1}{2})}$)	1.279	-0.0645 ± 0.0304‡*

* Significant at $0.01 < P < 0.05$. ** Significant at $0.001 < P < 0.01$. *** Significant at $P < 0.001$. † Simple regression coefficient. ‡ Partial regression coefficient.

In both experiments the amounts of adjustment were small, and after adjustment the differences between treatment means were still apparent at the same levels of significance.

The proximate compositions of the dried milks are given in Table 2; the amounts of fat, lactose, protein and ash in milk B did not differ markedly from those in the other two milks.

Table 2. Expts 1 and 2. Proximate composition of the spray-dried and roller-dried skim milks after reconstitution

	Expt 1		Expt 2		
	Spray-dried (milk A, 'severe' heat treatment)	Spray-dried (milk B, 'mild' heat treatment)	Spray-dried (milk A)	Spray-dried (milk B)	Roller-dried
Fat (g/100 g)	0.00	0.00	0.01	0.00	0.01
Lactose (anhydrous) (g/100 g)	4.95	4.82	5.19	5.07	4.81
Protein (g/100 g)	3.66	3.57	3.55	3.72	3.78
Ash (g/100 g)	0.83	0.85	0.89	0.86	0.88
Calcium (mg/100 g)	—	—	137	139	134
Phosphorus (mg/100 g)	—	—	104	106	102

The distribution of N in the roller-dried milk is shown in Table 3 and, for comparison, the N fractions expressed as percentages of the total N are given for one batch of each of the spray-dried milks used in an earlier experiment (Shillam *et al.* 1962*b*). Denaturation of the whey proteins and in particular the β -lactoglobulin appeared to be greater in the roller-dried milk than in the 'severely' heated spray-dried milk.

*Live-weight gain**Expt 3*

The mean live-weight gain/day of each calf during the 7-day experimental period is given in Table 4. Calves given milk B ('mild' heat treatment) gained a mean of 0.40 lb/day more weight than those given milk A ('severe' heat treatment).

Table 3. *Expt 2. Nitrogen partition of the roller-dried skim milk and comparison with that of spray-dried skim milk*

Nitrogen partition	Roller-dried skim milk* (mg/100 g milk)	N in fraction as a percentage of total N		
		Roller-dried skim milk	Milk A (spray-dried, 'severe' heat treatment)	Milk B (spray-dried, 'mild' heat treatment)
Total N	614	100.0	100.0	100.0
Casein N	536†	87.3	85.0	78.0
Non-casein N	78	12.7	15.0	22.0
Non-casein protein N	37	6.0	8.4	15.4
Total albumin N	11	1.8	4.9	11.1
β-lactoglobulin N	2	0.3	3.5	7.0
Residual albumin N	9	1.5	1.4	4.1
Proteose-peptone + globulin N	26	4.2	3.5	4.3
Non-protein N	41	6.7	6.6	6.6

* Reconstituted at the rate of 1 lb milk powder/9 lb water. † Includes denatured non-casein N.

Apparent digestibility

The results are given in Table 4.

Total solids. The mean apparent digestibility by the calves of the total solids in milk B (87.5%) was higher than that in milk A (82.0%). After differences due to treatments had been removed, the apparent digestibility was positively related to the dry-matter content of the faeces. The observed values were therefore adjusted by means of the regression coefficient of apparent digestibility of total solids on dry-matter content of the faeces given in Table 4. After adjustment, the mean value of apparent digestibility of the total solids for milk B was significantly greater ($0.01 < P < 0.05$) than for milk A.

Nitrogen. The mean apparent digestibility of N was higher in milk B (75.1%) than in milk A (62.7%). After adjustment of the apparent digestibility of N for differences in the dry-matter content of the faeces between treatments by means of the regression coefficient given in Table 4, the difference was significant at $0.01 < P < 0.05$.

N balance and the biological value of the protein

The results are summarized in Table 5. The mean amount of N retained by the calves given milk A was only 63% of that retained by those given milk B. This difference appears to be largely a reflection of treatment differences in the amounts of N apparently digested for, after adjustment of retention of N for differences in amounts of apparently digested N by means of the regression coefficient given in Table 5, the mean amounts of N retained by the two groups of calves were similar.

Table 4. *Expt 3. Apparent digestibility of the total solids and nitrogen of the spray-dried skim milks, together with the weight gains of the calves during the period 3-10 days of age*

Calf no.	Weight at beginning of digestibility trial (lb)	Mean amount of diet ingested/day (lb)	Mean live-weight gain/day (lb)	Dry-matter content of faeces (%)	Coefficient of apparent digestibility (%)	
					Total solids	N
Milk A ('severe' heat treatment)						
1	70.75	8.36	0.21	11.7	75.4	44.1
2	50.00	7.70	0.07	9.1	77.3	59.7
3	80.75	9.69	0.29	20.4	93.3	85.7
4	92.25	7.92	-0.07	15.0	82.0	61.2
Mean	73.44	8.42	0.13	14.1	82.0	62.7
Adjusted mean†	—	—	—	—	80.5‡	60.2‡
Milk B ('mild' heat treatment)						
1	79.75	9.69	1.00	14.5	93.1	84.7
2	69.50	8.62	0.21	8.8	79.8	66.3
3	76.00	9.69	0.32	12.7	90.7	78.9
4	85.75	9.69	0.57	13.3	86.4	70.5
Mean	77.75	9.42	0.53	12.3	87.5	75.1
Adjusted mean†	—	—	—	—	89.0‡	77.5‡

Regression coefficient of apparent digestibility of total solids on dry-matter content of faeces = $1.665 \pm 0.307^{**}$
 Regression coefficient of apparent digestibility of nitrogen on dry-matter content of faeces = $2.811 \pm 0.910^*$

* Significant at $0.01 < P < 0.05$. ** Significant at $0.001 < P < 0.01$.

† Adjusted for differences between treatments in dry-matter content of faeces.

‡ Difference between adjusted means significant at $0.01 < P < 0.05$.

Table 5. *Expt 3. Results of nitrogen balance tests with the spray-dried skim milks during the period 3-10 days of age*

Calf no.	Intake (g)	Excretion (g)		Balance (g/day)	Biological value of the protein†
		Faeces	Urine		
Milk A ('severe' heat treatment)					
1	144.2	80.6	52.3	1.61	53
2	134.9	54.4	34.2	6.61	76
3	169.6	14.3	82.5	8.96	60
4	133.3	51.7	45.1	5.21	75
Mean	145.5	52.8	53.6	5.60	66
Adjusted mean‡	—	—	—	7.11	—
Milk B ('mild' heat treatment)					
1	179.1	27.4	72.9	11.25	68
2	157.7	53.2	70.3	4.89	54
3	171.4	36.2	69.0	9.45	66
4	172.7	50.9	53.4	9.75	76
Mean	170.2	41.9	66.4	8.84	66
Adjusted mean‡	—	—	—	7.33	—

Regression of N balance/day on apparently digested N/day (N intake - faecal N) = $0.596 \pm 0.151^{**}$

** Significant at $0.001 < P < 0.01$.

† For calculation, see p. 178.

‡ Adjusted for differences between treatment means in amounts of apparently digested N.

The biological value of a protein, as defined by Mitchell (1923-4), is given by the equation

$$BV = 100 \times \frac{NI - (UN - EN) - (FN - MN)}{NI - (FN - MN)},$$

where BV = biological value, NI = nitrogen intake, UN = urinary N excretion, EN = endogenous N excretion, FN = faecal N excretion, MN = metabolic component of the faecal N. By substituting in this equation, the values for EN and MN obtained in studies with young calves by Blaxter & Wood (1951) and by Cunningham & Brisson (1957), mean biological values of the protein of the two diets were calculated. The values used were:

Reference	Endogenous N (mg/kg body- weight daily)	Metabolic faecal N (g/100 g dry matter ingested)
Blaxter & Wood (1951)	81.9	0.45
Cunningham & Brisson (1957)	65.3	0.334

Calculated thus, the biological values of the proteins of the two dried skim milks were similar (Table 5).

DISCUSSION

To be of maximum value, milk-substitute diets should be capable of supplying the calf's nutritional needs immediately after the colostrum-feeding period. The findings reported in this paper show that during the first 3 weeks of life, the quality of the milk powder included in a milk substitute may have a very marked effect on the performance of the calf. Thus in both Expts 1 and 2 the growth rates of calves given 6 pints of colostrum and then a diet containing the 'severely' preheated spray-dried milk was only 66% of that of calves given the spray-dried milk that had been subjected to only a 'mild' preheating treatment; this adverse effect on performance could not be rectified by a chlortetracycline supplement. The growth rate of calves given the roller-dried milk, which may be considered to have been severely heated, was only 72% of that of calves given the better-quality spray-dried product. The results of Expt 3 showed that the difference in weight gains was at least partly associated with a lowered apparent digestibility of N in the more severely heated spray-dried milk. However, after adjustment of the mean weight gains for between-treatment differences in the amounts of apparently digested N/day (by means of the regression coefficient of live-weight gain/day on apparently digested N, 0.051 ± 0.018 , significant at $0.01 < P < 0.05$), the mean daily gain of calves given milk B (0.40 lb) was still appreciably greater than that of the calves given milk A (0.26 lb). This finding supports the hypothesis put forward in an earlier publication (Shillam, Dawson & Roy, 1960) that the depression in weight gain that occurs as a result of heat treatment of the diet is not solely accounted for by a quantitative reduction in the amount of available protein. Thus in the work described here, if it is assumed that the denatured whey proteins are resistant to enzymic hydrolysis, the loss to the calf was only about 10% of the total protein in the diet. Loss or changes in availability of certain constituent amino acids of the denatured protein may be involved, and this possibility is being

investigated. However, from the finding reported now that heat treatment had little or no effect on the utilization of the apparently digested N or on the biological value of the proteins, it would appear that a shortage of available amino acids is unlikely to be very marked.

There is considerable evidence from experiments in which the rat has been used as an experimental animal to show that the drying process may affect the nutritive value of milk proteins. We have already commented on the work of Fairbanks & Mitchell (1935), Henry, Houston, Kon & Osborne (1939) and Henry & Kon (1947-8) on the effect of the preheating treatment of spray-dried milk (Shillam *et al.* 1962*b*). With roller-dried milk, Nevens & Shaw (1933) demonstrated that the digestibility of the proteins was significantly lower than that obtained with fresh milk. Miyawaki, Kanazawa & Kanda (1932) found that the digestibility of the proteins of roller-dried milk was slightly but significantly lower than of those of spray-dried milk, and similar but more pronounced differences between both digestibility and biological values of the proteins of spray- and roller-dried whey have been found also by DeBaun & Connors (1954) and Riggs, Beaty & Mallon (1955). On the other hand, Henry *et al.* (1939) were unable to detect any difference in the nutritive value of the proteins of milk dried by the two methods. This variation in reported findings is undoubtedly a result of differences in the time-temperature relationships employed, since Richter & Schiller (1956) obtained biological values of 86-97 for a series of spray-dried milks and of 80-96 for a series of roller-dried powders, the lower values for the latter milk being associated with higher processing temperatures. Earlier Fairbanks & Mitchell (1935) had shown that the biological value of a 'specially prepared' low-temperature roller-dried powder was similar to that of raw liquid milk and to that of a non-preheated spray-dried powder, whereas those for commercially prepared roller- and spray-dried powders were depressed, with a loss in nutritive value in a slightly scorched roller powder of more than 20%. The extensive studies of Fink and his colleagues (Fink, Schlie & Ruge, 1958, 1959) on the effect of heat treatment of milk on the incidence of fatal liver necrosis in rats is discussed in a later paper in this series (Shillam & Roy, 1963).

The validity of the use of the values obtained by other workers for endogenous N to calculate the biological values of the proteins can be determined from the equation relating daily excretion of urinary N (UN) to daily intake of apparently digested N (ADN):

$$UN = 8.57 + 0.403 (ADN - 15.79).$$

Thus when ADN is zero, the value for UN, which is then an estimate of endogenous N, is 64.4 mg/kg body-weight daily and corresponds very closely to the value of 65.3 obtained by Cunningham & Brisson (1957) and is somewhat less than the value of 81.9 obtained by Blaxter & Wood (1951).

Our findings suggest that whenever possible milk substitutes for young calves should contain dried milk in which little or no denaturation of the whey proteins has occurred.

SUMMARY

1. Ninety-two newborn calves were each given 6 pints of colostrum and were used in three experiments. The first two experiments compared the effect of diets containing spray-dried skim milks that had been submitted to either a 'mild' (77° for 15 sec) or a 'severe' (74° for about 30 min) preheating treatment during the drying process; in the second experiment milk dried by the roller process was compared with the two spray-dried powders, and the effect of chlortetracycline supplementation of the spray-dried milks was studied. The third experiment comprised a series of digestibility and balance trials with the two spray-dried milks.

2. The mean weight gain of calves given the 'severely' preheated spray-dried milk was 66%, and the mean weight gain of those given the roller-dried milk was 72%, of that of calves given the spray-dried milk subjected to the 'mild' heat treatment. The differences were highly significant. The mean incidence of scouring tended to be low and did not differ between treatments. The chlortetracycline hydrochloride supplement, 125 mg/day, had no effect on the incidence of scouring or on weight gain.

3. The mean apparent digestibility of the N in milk B (spray-dried, 'mild' heat treatment) was greater than that in milk A (spray-dried, 'severe' heat treatment). There were no differences between treatments in the utilization of the apparently digested N or in the biological value of the proteins.

4. There were no marked differences between the milks in their contents of fat, lactose, protein and ash.

5. Analyses of the N fractions of the roller-dried milk when compared with those of batches of the spray-dried milks used in an earlier study showed that about 61% of the non-casein proteins in the roller-dried milk appeared to have been denatured. The earlier studies showed that none of the non-casein proteins in milk B and 45% of those in milk A appeared to have been denatured.

6. The possibility is discussed that the lowered growth rate may be related to denaturation of the non-casein proteins, and it is suggested that milk-substitute diets for calves should contain skim-milk powder in which the non-casein proteins have not been denatured.

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