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

4*. Further studies on the effects of the preheating treatment of spray-dried skim milk and of ultra-high-temperature treatment

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Earlier experiments in this series have shown that diets containing certain heat-treated milks are less suitable for the well-being of the young calf than diets containing milk that had been given less severe or no heat treatment (Shillam, Dawson & Roy, 1960; Shillam, Roy & Ingram, 1962*a*, *b*). The experiment reported now was made to obtain further evidence on the detrimental effect of heat-treated milks and its association with the denaturation of a large proportion of the whey-protein fraction.

The skim-milk powder that had been preheated at 77° for 15 sec before spray-drying (milk B) and in which no apparent denaturation of the whey proteins had occurred (Shillam *et al.* 1962*b*) was reconstituted and subjected to ultra-high-temperature (UHT) treatment (135° for 1–3 sec); its nutritive value for the calf was then compared with that of untreated reconstituted milk B. In addition, whey proteins of milk B were added to the skim-milk powder that had been preheated at 74° for about 30 min before spray-drying (milk A) to find if the performance of calves given milk A could be improved and, if so, whether the growth rate of calves given this diet would be similar to that of calves given milk B. Since an earlier experiment (Shillam *et al.* 1962*b*), made under conditions of moderate to high 'infection' (see Roy, Palmer, Shillam, Ingram & Wood, 1955; Ingram, Shillam, Hawkins & Roy, 1958), had not shown that the weight gains of calves given milk A were less than those of calves given fresh separated milk, a further group of calves was given a diet containing fresh separated milk.

METHODS

Plan of experiment

The experiment was done in the autumn and winter months of 1957 after the calf house had remained empty for a period of 3 months during the summer. A randomized block design was used with five treatments in each of sixteen blocks as shown on p. 594.

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Eleven of the blocks consisted of Ayrshire bull calves and five of Shorthorns. Management of the calves throughout the experiment was as described earlier (Shillam *et al.* 1962*a*), except that the calves were pail-fed twice daily from birth instead of three times daily during the first 10 days of life. The mean daily live-weight gain was obtained by calculating the mean of the linear regression and of the difference between the 21st-day and 1st-day weights divided by 20 (see Roy, Shillam, Hawkins & Lang, 1958).

Treatment no.	Colostrum	Milk
17	400 ml or 600 ml separated colostrum	Milk A (dried skim milk ('severe' heat treatment) containing 2 % fat)
18		Milk A plus whey fraction of milk B (for amount added, see below)
19		Milk B (dried skim milk ('mild' heat treatment) containing 2 % fat)
20		UHT-treated milk B containing 2 % fat
21		Fresh separated milk containing 2 % fat

Diets

Colostrum

Each calf in the first ten blocks of the experiment was given as its first meal 400 ml separated colostrum, consisting of 100 ml from each of four different batches. As calves in the seventh and eighth blocks tended to have a high incidence of scouring after they had passed their meconium, the contents of coliform organisms in samples of the four batches of colostrum were determined. It was found that two of the colostrum samples contained 10^6 coliforms/ml. As a result, all batches of colostrum given to calves in blocks subsequent to the eighth were first heated at 63° for 30 min. As this heat treatment denatured about 10–15 % of the non-casein protein nitrogen, the amount of separated colostrum given to these calves was increased to 600 ml, consisting of 100 ml from each of six different batches of colostrum.

Milk

The diets given to calves on treatments 17, 19 and 21 were prepared as described earlier (Shillam *et al.* 1962*a*, *b*). The diet given to calves on treatment 20 was prepared by sterilizing reconstituted milk B at 135° for 1–3 sec: the operation of the UHT plant was as described previously (Shillam *et al.* 1960). About 40 gal were sterilized at any one time, and in all nine batches of UHT-treated milk were prepared. The milk was stored in 1 pint waxed cartons at −25° until required. The amount of milk required daily was thawed slowly and 1 lb of a milk of 20 % fat content (Shillam *et al.* 1962*a*) was added to each 9 lb of milk. The diets given to calves on treatment 18 were prepared in the following way. After reconstitution of milk B, 9 ml commercial (Hansen (Chr.) Laboratory Ltd) rennet/10 lb milk were added and the subsequent casein clot was separated from the whey by passing the mixture through a muslin cloth. The clot was discarded and the whey was dialysed with water to remove lactose. Cellophane bags varying from $\frac{1}{4}$ to 6 in. in diameter, and various methods of suspension and agitation of the bags in water, were used but it was only possible during a period of 24 h to reduce the lactose content in the large amount of whey required daily to 3·5 % from about 5 % in the original whey. The first four calves on treatment 18 were given a diet containing milk A reconstituted with either the 'dialysed' or undialysed whey from milk B instead of with water: the diet given to the remaining twelve calves on this treatment contained milk A, reconstituted with undialysed whey from milk B and water in the ratio of 1 part of whey: 2 parts of water. To each 9 lb of these mixtures, 1 lb of the milk of 20% fat content was added. The diet of the last twelve calves thus contained about 1·4% added lactose and 0·2% added whey proteins. Before the whey was used in the reconstitution of milk A, it was heated at 55° for 10 min to inactivate any rennet present.

The usual daily supplement of 3500 i.u. vitamin A in the form of halibut-liver oil concentrate and 700 i.u. synthetic vitamin D_3, dissolved in arachis oil was given to each calf.

The diets were given at a rate that had been found adequate for a growth rate of 1 lb/day when whole milk of 3·3% fat content was used (Roy *et al.* 1958).

Analytical methods

The partition of nitrogen was determined by the method of Aschaffenburg & Drewry (1959) on 1 pint samples from each of six batches, taken at random from the nine batches of UHT-treated milk prepared.

RESULTS

Mortality. The results are given in Table 1. Ten of the twenty-eight calves given diets containing milk A died, compared with eight of the thirty-two given either untreated milk B or UHT-treated milk B and one of the sixteen given fresh separated milk.

Of the calves that died, those given milk that had been subjected to the more severe heat treatments tended to succumb at an earlier age than those given milk that had received less drastic heat treatment.

Autopsy findings. The post-mortem findings are given in Table 2. Of the nineteen deaths, seventeen were associated with an *Escherichia coli* infection. Sixteen of the deaths were associated with an *Escherichia coli* localized intestinal infection and one with an *E. coli* septicaemia.

Performance of surviving calves. Two of the first four calves that were given milk A reconstituted with undiluted whey from milk B (treatment 18) survived the 3-week experimental period; their high rate of weight gain (mean of 0·97 lb/day) may have been associated with the large amount of lactose in the diet. The results of the performance of the four calves given this diet were therefore not included in the general analysis of the experiment.

The results given in Table 1 show that the observed mean weight gain of calves given milk B was greater than that of calves given milk B that had been subjected to UHT treatment and greater than that of calves given milk A. The mean rate of weight gain of calves given milk A containing whey from milk B was greater than that of calves given milk A alone and greater, but not significantly so, than that of calves given milk B. The mean weight gain of calves given fresh separated milk was greater than

Table 1. *Effect of 'severe' (milk A) or 'mild' (milk B) preheating treatment of spray-dried skim milk and of UHT treatment on the performance of calves (mean values with their standard errors)*

(All diets contained 2 % margarine fat)

	Treatment no. and diet					
	17 Milk A	18 Milk A plus whey from milk B†	19 Milk B	20 Milk B subjected to UHT treatment	21 Fresh separated milk	Significance of difference between treatments
Calves:						
No. used	16	12	16	16	16	—
No. died	5	5	4	4	1	—
Age at death (days)	6 ± 1·9	5 ± 0·7	10 ± 1·9	5 ± 0·6	14	—
Live-weight gain/day of surviving calves (lb)	0·20 ± 0·08	0·66 ± 0·10	0·56 ± 0·07	0·34 ± 0·07	0·45 ± 0·07	18 > 17**, 18 > 20**, 19 > 17**, 19 > 20*, 21 > 17*
Birth weight of surviving calves (lb)	77·0 ± 3·4	86·3 ± 4·3	76·0 ± 3·3	70·5 ± 3·3	74·1 ± 2·9	—
Milk consumption of surviving calves (pints)	129·6 ± 5·8	146·4 ± 7·2	141·0 ± 5·5	126·8 ± 5·5	134·7 ± 4·9	—
No. of days on which surviving calves scoured	4 (range 0–9)	3 (range 0–7)	3 (range 0–8)	2 (range 0–7)	4 (range 1–10)	—
No. of days on which surviving calves had a high rectal temperature (> 102·8 °F)	3 (range 0–12)	3 (range 0–4)	3 (range 1–9)	3 (range 0–9)	3 (range 0–11)	—
Adjusted live-weight gain/day of surviving calves (lb)‡	0·27 ± 0·05	0·66 ± 0·06	0·49 ± 0·04	0·37 ± 0·04	0·43 ± 0·04	18 > 17***, 18 > 19**, 18 > 20***, 18 > 21***, 19 > 17*, 21 > 17*
Time between birth and complete passage of meconium (h)	39·5 ± 3·3	34·7 ± 4·3	42·9 ± 3·1	36·7 ± 3·0	41·5 ± 3·4	—

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

† Milk A reconstituted with whey from milk B and water in the ratio of 1:2.

‡ Adjusted for differences between treatment groups in mean birth weight and total milk consumption.

that of calves given milk A. Most of the detrimental effect on weight gain of calves given diets containing milk A or milk B subjected to UHT treatment appeared to occur during the first 10–12 days of life, even though the incidence of scouring of calves on these treatments was no greater than that of calves on the remaining treatments.

Table 2. *Number of calves that died, classified according to post-mortem findings*

Post-mortem finding		Treatment no. and diet: 17 Milk A	18 Milk A + whey from milk B	19 Milk B	20 Milk B subjected to UHT treatment	21 Fresh separated milk
Escherichia coli	Localized intestinal infection	5	5	2	4	—
E. coli and *Pasteurella septica*	Septicaemia and polyserositis	—	—	1	—	—
	Rumenitis*	—	—	1	—	1

Milks A and B, spray-dried skim milk prepared with a 'severe' (A) or 'mild' (B) preheating treatment. All diets contained 2 % margarine fat.

* Probably calf diphtheria associated with *Fusiformis* sp. infection.

When the mean daily gains were analysed by multiple covariance and adjusted for differences between treatment groups in mean birth weight and total milk consumption the differences between treatment means were still significant with the exception of that between treatment 19 (milk B) and treatment 20 (milk B, UHT-treated) for which the t value was 1·93 ($t = 2·07$ at $P = 0·05$). The adjusted mean weight gain of calves given milk A containing whey from milk B (treatment 18) was significantly greater than that of calves on all the other treatments. The relevant partial regression coefficients are given below, the adjusted mean values are given in Table 1.

	General mean	Partial regression coefficient with its standard error
Live-weight gain/day (lb)	0·426	—
Birth weight (lb)	75·79	$-0·0130 \pm 0·0020$***
Total milk consumption (pints)	134·80	$+0·0118 \pm 0·0012$***

*** Significant at $P < 0·001$.

There were no significant differences between treatments in the incidence of scouring or of a high rectal temperature. Calves given milk B or fresh separated milk tended to pass their meconium more slowly than those given the diets containing milk that had been subjected to the more severe heat treatments.

Partition of nitrogen. Table 3 shows that approximately 55 % of the non-casein proteins in milk B was denatured by UHT treatment and that the amount of non-casein protein in milk A was only 56 % of that present in milk B. Of the non-casein protein N, about 90 % of the β-lactoglobulin N and 20 % of the residual albumin N were denatured by the UHT process. Approximately 85 % of the globulin N was

Table 3. *Nitrogen partition of the skim milks*

	Milk B ('mild' preheating treatment) subjected to UHT-treatment								Untreated milk B		Milk A ('severe' preheating treatment)	
	Batch no. (mg/100 g milk)						Mean					
	2	4	5	7	8	9	mg/100 g milk	As a % of total N	mg/100 g milk	As a % of total N	mg/100 g milk	As a % of total N
Total N	516	494	549	568	566	546	540	100·0	559	100·0	573	100·0
Casein N*	450	418	471	493	490	476	466	86·3	436	78·0	487	85·0
Non-casein N	66	76	78	75	76	70	74	13·7	123	22·0	86	15·0
Non-casein protein N	31	40	42	37	37	32	37	6·9	86	15·4	48	8·4
Total albumin N	21	20	22	23	21	22	22	4·1	62	11·1	28	4·9
β-Lactoglobulin N	4	3	4	4	3	3	4	0·7	39	7·0	20	3·5
Residual albumin N†	17	17	18	19	18	19	18	3·3	23	4·1	8	1·4
Proteose–peptone + globulin N	10	20	20	14	16	10	18	3·3	24	4·3	20	3·5
Non-protein N	35	36	36	38	39	38	37	6·9	37	6·6	38	6·6

* Includes denatured non-casein protein N.

† Sum of α-lactalbumin, 'blood' serum albumin and two minor components (see Aschaffenburg & Drewry, 1959).

denatured, but since the globulin N value was obtained by difference from two rather large values, a composite figure for proteose–peptone N and globulin N is given in Table 3.

DISCUSSION

The results of this experiment, made under conditions of moderate 'infection', show clearly that, as judged by the rate of weight gain of the young calf, the nutritive value of dried skim milk appeared to be unaffected when the liquid milk was heated at 77° for 15 sec before drying, whereas milk heated at a slightly lower temperature for about 30 min before drying was inferior to unheated separated milk. Thus the difference in rate of weight gain of calves given the dried skim-milk diets observed in an earlier experiment (Shillam *et al.* 1962*b*) has been confirmed, but in contrast to findings in the earlier experiment, in the study now described the weight gain of calves given fresh separated milk was as expected. Although the digestive disturbances, as indicated by the incidence of scouring and the time interval between birth and the complete passage of the meconium, tended to be high in calves given the 'severely' preheated dried skim milk A, it is clear that even when allowance was made for the reduction in milk intake that occurred as a result of scouring, the differences in performance between these calves and calves given milk B or fresh separated milk were still apparent.

Table 4. *Relationship between denaturation of the non-casein proteins of the skim milks and weight gains of the calves*

	Nitrogen fraction as a percentage of that in milk B		
	Milk A ('severe' preheating treatment)	UHT-treated milk B	Milk B ('mild' preheating treatment)
β-Lactoglobulin N	50	10	100
Residual albumin N	35	80	100
Globulin N	66	15	100
Proteose–peptone N	102	97	100
Adjusted live-weight gain/day of calves (lb)*	0·27	0·37	0·49

* See Table 1.

As a result of the findings in earlier experiments it was suggested that the poor performance of calves given milk A may have been associated with denaturation of part of the whey-protein fraction of this milk. Although other factors may also be involved, the findings now described appear to support this view. However, the reduction in weight gain caused by the UHT treatment of milk B (0·12 lb/day) was less than the difference in weight gain between calves given milk A and those given milk B (0·22 lb/day), even though the amount of non-casein proteins denatured by the UHT treatment was greater than the difference in non-casein protein content between milk A and milk B (Table 3). Thus milk A contained about 56 % and UHT-treated milk B about 45 % of the non-casein protein N in untreated milk B. From Table 4, which gives the protein fractions in milk A and in UHT-treated milk B as a percentage of those in untreated milk B, it can be seen that the residual albumins appear to be the only fraction denatured to a greater extent by the heat treatment of the milk before drying than by UHT treatment. These findings could be interpreted as indicating that denaturation of the residual albumin fraction was at least partly associated with the lowered performance of the calves. However, in a later experiment already reported, Shillam *et al.* (1960) found that UHT treatment of whole milk denatured about 62 % of the residual albumin N compared with 20 % in the study described here.

A feature of the experiment now described was the relatively high rate of weight gain of calves given milk A to which had been added the whey fraction of milk B. There is little doubt that the increased intake of calories was associated with part of this growth response, but by calculation it is possible to show that the added calories from the lactose and protein did not appear to account for all of the response. Each 10 lb of diet given to calves on treatment 18 contained 2·7 lb added whey, or about 0·14 lb lactose and 0·02 lb non-casein proteins. The gross energy of this added whey was thus about 250 kcal from the lactose and 50 kcal from the protein/10 lb diet, or a total of about 37·5 kcal/pint. Roy *et al.* (1958) have calculated that in the absence of scouring and under conditions of low 'infection' it was necessary for calves given a diet containing milk A together with 2 % fat and 2·5 % glucose to ingest about 1250 kcal above their maintenance requirement for every 1 lb/day gain in weight. The mean daily milk intake of calves on treatment 18 was 7 pints, and thus they ingested about 250 additional kcal/day from the whey; this amount appears to be sufficient to give an additional live-weight gain of 0·2 lb/day, whereas the difference in mean live-weight gain/day between calves on treatment 18 and those given milk A alone was 0·39 lb (Table 1). It is improbable that more than a fraction of the extra response of 0·19 lb/day could have been associated with the higher content of minerals and water-soluble vitamins in the diet given to calves on treatment 18. As results from a later experiment (Shillam & Roy, 1963) showed no marked differences in the calorific values of the two spray-dried milks, evidence can be adduced that our estimated increase in live-weight gain of calves in treatment 18 was unlikely to be due entirely to the additional calories ingested. In the first instance, if the gain of 0·2 lb/day presumed to be associated with the extra calorie intake is subtracted from the mean live-weight gain of 0·66 lb/day of calves given the extra calories in the form of whey, the remaining figure of 0·46 lb/day is very similar to that of 0·49 lb/day for calves given milk B (treatment 19). Secondly, if that part of the increase in weight gain not explained by the extra intake of calories, i.e. 0·19 lb/day, is added to the mean daily gain of 0·27 lb of calves given milk A alone, the resulting figure is very similar to the mean daily gain of calves given milk B.

Thus, from the results of this experiment it is apparent that the addition of whey to a milk that contained a large proportion of denatured whey proteins (milk A) gave a diet that increased the growth rate of calves to a level which, after deduction of weight gain due to the extra calories given, was equal to that of calves given milk containing no denatured protein (milk B). These findings suggest strongly that the low growth rate of calves given the more severely heat-treated milks was associated with the denaturation of a large proportion of the whey proteins in these milks. More conclusive evidence to support this view would have been forthcoming if a further treatment had been included in which a similar amount of whey had been added to milk B; such a diet might have given an increase in growth rate that could have been entirely accounted for by the additional calories ingested.

SUMMARY

1. Eighty newborn calves were used in an experiment to find the effect on performance of the preheating treatment of dried skim milk and of UHT-treatment.

2. Under conditions of moderate 'infection', the mean daily weight gain of calves given a spray-dried skim milk preheated at 77° for 15 sec before drying (milk B) was similar to that of calves given fresh separated milk and significantly greater than that of calves given a spray-dried skim milk preheated at 74° for about 30 min (milk A) or milk B subjected to UHT treatment (135° for 1–3 sec) after reconstitution.

3. Addition of the whey fraction of milk B to milk A gave a growth response that appeared unlikely to be entirely due to the extra intake of calories.

4. UHT treatment of milk B denatured about 55% of the whey proteins.

5. Mortality rates on the various treatments were not significantly different.

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