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

6*. The effect of the addition of calcium

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It is well established that calcium is associated with the rennet coagulation of milk, and that when milk is heated the concentration of soluble and ionizable calcium decreases by up to 25% (Lampitt & Bushill, 1934; Verma & Sommer, 1950; Bernadoni & Tuckey, 1950; Harman & Slatter, 1950; Hilgeman & Jenness, 1951; Baker, Gehrke & Affsprung, 1954; Christianson, Jenness & Coulter, 1954; Hostettler & Stein, 1958; Davies & White, 1959). It thus seemed possible that the poor performance of calves given diets containing milks that had been prepared with severe heat treatments (Shillam, Dawson & Roy, 1960; Shillam, Roy & Ingram, 1962*a–c*; Shillam & Roy, 1963) may have been associated with changes in the physical nature of the rennet clot formed in the abomasum. Tests were made in vitro with the skim milk that had been preheated at a temperature of 74° for about 30 min before being spray-dried (Shillam *et al.* 1962*a*); they showed that this milk when reconstituted required the addition of 500 mg calcium (as $CaCl_2.6H_2O$)/l. to produce a clot with rennet (1 ml commercial rennet (Hansen (Chr.) Laboratory Ltd)/500 ml reconstituted milk) similar in appearance to that obtained with raw separated milk. Without the addition of calcium, the dried skim milk showed no clotting during the first 3 h after the addition of rennet and only a very finely flocculated curd after 8 h.

Two experiments with newborn calves were made. In the first, the effects on mortality rate of adding calcium to diets containing the 'severely' heat-treated spray-dried milk, ultra-high-temperature-treated (135° for 1–3 sec) milk or raw separated milk were compared. No beneficial effect of calcium on mortality rate was observed but, since any effect on the rate of weight gain may have been masked by the high level of 'infection' (see Roy, Palmer, Shillam, Ingram & Wood, 1955) in the calfhouse at the time when the experiment was made, the second experiment was done under conditions of low to moderate 'infection', and a comparison was made of the effects on the rate of weight gain of the calf of the addition of calcium to diets containing the spray-dried milks prepared with different preheating treatments.

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METHODS

Plan of experiments

Both experiments were of randomized block design. Expt 1 was done in the spring of 1956 after 118 calves, many of which died, had passed through the calfhouse after a period of vacancy during the previous summer. Twenty-three Ayrshire and nineteen Shorthorn bull calves were used. Treatments 30, 31 and 32 were omitted from the last two blocks of the experiment owing to a shortage of calves. The plan was:

Treatment no.	Colostrum	Milk	Calcium given
29	400 ml separated colostrum	Raw separated milk containing 2 % fat	None
30	400 ml separated colostrum	Raw separated milk containing 2 % fat	500 mg/l. diet
31	400 ml separated colostrum	Milk A (spray-dried skim milk, 'severe' heat treatment, containing 2 % fat)	None
32	400 ml separated colostrum	Milk A (spray-dried skim milk, 'severe' heat treatment, containing 2 % fat)	500 mg/l. diet
33	400 ml separated colostrum	UHT-treated separated milk containing 2 % fat	None
34	400 ml separated colostrum	UHT-treated separated milk containing 2 % fat	500 mg/l. diet

Expt 2 was done during the autumn of 1959. Twenty Shorthorn and twenty Ayrshire bull calves were used. There were four treatments in each of ten blocks:

Treatment no.	Colostrum	Milk	Calcium given
35	6 pints whole colostrum	Milk A (spray-dried skim milk, 'severe' heat treatment, containing 2 % fat)	None
36	6 pints whole colostrum	Milk A (spray-dried skim milk, 'severe' heat treatment, containing 2 % fat)	500 mg/l. diet
37	6 pints whole colostrum	Milk B (spray-dried skim milk, 'mild' heat treatment, containing 2 % fat)	None
38	6 pints whole colostrum	Milk B (spray-dried skim milk, 'mild' heat treatment, containing 2 % fat)	500 mg/l. diet

Calves

With a few minor exceptions, the collection, housing and management of the calves were as in earlier experiments (Shillam *et al.* 1962*a*).

Diets

Basal diets. The basal diets were prepared by the methods described earlier (Shillam *et al.* 1962*a–c*); milk A had been preheated at a temperature of 74° for about 30 min ('severe' heat treatment) and milk B at 77° for 15 sec ('mild' heat treatment) before being spray-dried.

Colostrum. Each calf was given in Expt 1 400 ml separated colostrum and in Expt 2, 3410 ml (6 pints) whole colostrum collected, stored and given in the usual manner (Shillam *et al.* 1962*a*; Shillam & Roy, 1963).

Calcium. A solution containing 410 g $CaCl_2.6H_2O$/l. was prepared. Of this solution 30·3 ml were added to each 10 lb of the diets given to calves on treatments 30, 32, 34, 36 and 38; these diets thus contained 500 mg added calcium/l. The calves on the other treatments were given an additional 30·3 ml water/10 lb diet.

Measurements of abomasal contents

Surviving calves in Expt 2 were slaughtered about 4½ h after the morning feed on the 21st day of life and their abomasums removed. Within 1 h the abomasum was cut open and the total contents were removed. The amounts of curd and whey present were recorded after the curd had been roughly broken up and the whey allowed to drain for 10 min through a B.S. wire mesh no. 36. The dry-matter content of the curd was obtained by drying to constant weight at 100°.

RESULTS

Mortality

The results are given in Tables 1 and 2. In Expt 1, six of the fourteen calves given the diet containing raw separated milk died compared with ten of the twelve given the 'severely' heated dried skim milk and ten of the sixteen given the UHT-treated milk. The addition of calcium had no effect on mortality rate; twelve of twenty calves given added calcium died compared with fourteen of twenty-two given no additional calcium. The age at which death occurred tended to be greater for calves given raw separated milk than for those given the dried skim milk; the addition of calcium appeared to have no effect in raising the age at death. In Expt 2, which was made under conditions of lower 'infection', only two calves died.

The deaths of twenty-four of the twenty-six calves that died in Expt 1 and of the two calves that died in Expt 2 were associated with an *Escherichia coli* localized intestinal infection. One death in Expt 1 was associated with an *E. coli* septicaemia and the other with 'milk bloat'.

Performance of surviving calves

Expt 1. Owing to the high 'infection', calves that survived the experimental period scoured badly and their live-weight gains were poor (Table 1). There were no differences in weight gain between the calves given the different basal diets, nor did the addition of calcium have an effect on growth rate, incidence of scouring or a high rectal temperature or the time taken by each calf to pass its meconium completely. Adjustment of mean daily weight gains for differences between treatment groups in birth weight and milk consumption showed no significant differences between treatment means. The partial regression coefficients used for the adjustment are given below; the adjusted mean values are given in Table 1.

	General mean	Partial regression coefficient with its standard error
Live-weight gain (lb/day)	−0·043	—
Birth weight (lb)	80·71	−0·0318 ± 0·0052***
Milk consumption (pints)	103·75	+0·0257 ± 0·0030***

*** Significant at $P < 0·001$.

Expt 2. The addition of calcium appeared to have a slight, though not significant, beneficial effect on mean weight gains during the first 3 weeks of life (Table 2).

Table 1. *Expt 1. Performance of calves (mean values with their standard errors or ranges) given diets containing a 'severely' preheated (74° for about 30 min) spray-dried skim milk, a UHT-treated (135° for 1–3 sec) separated milk or a raw separated milk with and without additional calcium*

	Treatment no. and diet					
	29 Raw separated milk containing 2 % fat	30	31 Spray-dried skim milk containing 2 % fat	32	33 UHT-treated separated milk containing 2 % fat	34
	No calcium	500 mg calcium/l.	No calcium	500 mg calcium/l.	No calcium	500 mg calcium/l.
Calves						
No. used	8	6	6	6	8	8
No. died	3	3	5	5	6	4
Age at death (days)	12 ± 1·2	7 ± 2·5	8 ± 2·2	6 ± 1·0	6 ± 0·6	11 ± 2·6
Live-weight gain/day of surviving calves (lb)	−0·06 ± 0·13	−0·05 ± 0·19	−0·28†	−0·03†	0·11 ± 0·23	−0·09 ± 0·15
Birth weight (lb)	81·7 ± 5·6	87·1 ± 7·2	—	—	68·9 ± 8·9	80·7 ± 6·3
Milk consumption (pints)	106·0 ± 9·5	113·5 ± 12·3	—	—	90·0 ± 15·1	100·5 ± 10·6
No. of days on which surviving calves scoured	8 (range 5–11)	9 (range 5–13)	8†	4†	10 (range 8–11)	8 (range 5–13)
No. of days on which surviving calves had a high rectal temperature (> 102·8° F)	2 (range 1–2)	1 (range 0–2)	0†	0†	1 (range 0–1)	1 (range 0–1)
Mean time between birth and complete passage of meconium (h)	32·7 ± 1·5		29·7 ± 1·5		31·4 ± 1·5	
Adjusted live-weight gain/day of surviving calves (lb)‡	−0·09 ± 0·04		§		0·02 ± 0·05	

† One calf only.
‡ Adjusted for differences between treatment groups in mean birth weight and milk consumption.
§ Values not included in analysis of multiple covariance.

Table 2. *Expt 2. Performance of calves (mean values with their standard errors or ranges) given diets containing spray-dried skim milk prepared with either a 'severe' or 'mild' preheating treatment and with and without additional calcium*

	Treatment no. and diet				
	35	36	37	38	
	Milk A ('severe' heat treatment)		Milk B ('mild' heat treatment)		
	No calcium	500 mg calcium/l.	No calcium	500 mg calcium/l.	Significance of difference between treatments
Calves					
No. used	10	10	10	10	—
No. died	0	0	0	2	—
Live-weight gain/day of surviving calves (lb)					
(*a*) during first 3 weeks of life	0·17 ± 0·07	0·23 ± 0·07	0·41 ± 0·07	0·45 ± 0·08	35 + 36 < 37 + 38**†
(*b*) during first 12 days of life	−0·06 ± 0·12	0·22 ± 0·12	0·43 ± 0·12	0·57 ± 0·13	35 + 36 < 37 + 38**†
Birth weight (lb)	75·8 ± 3·3	79·2 ± 3·3	80·0 ± 3·3	69·4 ± 3·7	—
Milk consumption (pints)					
(*a*) during first 3 weeks of life	137·7 ± 4·5	148·7 ± 4·5	149·4 ± 4·5	140·8 ± 5·0	—
(*b*) during first 12 days of life	75·4 ± 8·0	83·9 ± 8·0	82·6 ± 8·0	77·8 ± 8·9	—
'Occupation time' (Roy *et. al.* 1955) (log days)	1·703 ± 0·154	1·794 ± 0·154	1·636 ± 0·154	1·721 ± 0·173	—
No. of days on which surviving calves scoured	3 (range 0–9)	3 (range 0–7)	3 (range 0–6)	3 (range 0–5)	—
No. of days on which surviving calves had a high rectal temperature (> 102·8° F)	3 (range 0–4)	2 (range 0–7)	2 (range 0–4)	1 (range 0–4)	—
Time between birth and complete passage of meconium (h)	44·4 ± 4·6	51·5 ± 4·9	41·3 ± 4·9	42·3 ± 5·8	—
Curd: whey ratio of abomasal contents at 3 weeks of age (parts curd/100 parts whey)	104 ± 34‡	151 ± 32§	164 ± 41‖	211 ± 34‡	—
Dry-matter content of abomasal curd (%)	22·1 ± 1·1‡	23·6 ± 1·0§	28·5 ± 1·3‖	25·5 ± 1·1‡	35 + 36 < 37 + 38**†
Adjusted live-weight gain/day (lb)					
(*a*) during first 3 weeks of life¶	0·25 ± 0·05	0·23 ± 0·05	0·38 ± 0·05	0·39 ± 0·05	35 + 36 < 37 + 38**†
(*b*) during first 12 days of life¶	0·06 ± 0·08	0·21 ± 0·08	0·41 ± 0·08	0·47 ± 0·09	35 + 36 < 37 + 38***†

** Significant at $0.001 < P < 0.01$.
† Two treatment nos. joined by a plus sign denote the mean value for those treatments.
§ Eight calves only.
¶ Adjusted for differences between treatment groups in mean birth weight, milk consumption and log 'occupation time'.
*** Significant at $P < 0.001$.
‡ Seven calves only.
‖ Five calves only.

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However, when the mean daily weight gains were analysed by multiple covariance and adjusted for differences between treatment groups in mean birth weight, milk consumption and log 'occupation time' (see Roy *et al.* 1955) this trend was no longer apparent. There was a highly significant difference in weight gains between the groups of calves given the spray-dried skim milks that had been prepared with different pre-heating treatments. This finding confirms the results of an earlier experiment made under conditions of low 'infection' (Shillam & Roy, 1963). From an examination of the daily live weights it appeared that the difference in growth rate between the skim milks tended to be most marked during the first 12 days of the experimental period. Further, the improvement in weight gain resulting from the addition of calcium to milk A ('severe' heat treatment), although not significant, appeared to be twice as great as that obtained with calcium supplementation of milk B during this time. A multiple covariance analysis of live-weight gain/day during the first 12 days of life on birth weight, milk consumption and log 'occupation time' was therefore made. From the adjusted mean values given in Table 2, it can be seen that the poor performance of calves given milk A was, in fact, most marked during the first 12 days of life and that an appreciable, though not statistically significant, improvement in growth rate resulted from the supplementation of this milk with calcium.

The partial regression coefficients used in the adjustments were:

	General mean		Partial regression coefficient with its standard error	
	First 3 weeks	First 12 days	First 3 weeks	First 12 days
Live-weight gain/day (lb)	0·306	0·277	—	—
Birth weight (lb)	76·46	76·46	−0·0159 ± 0·0036***	−0·0247 ± 0·0051***
Milk consumption (pints)	144·32	80·03	0·0143 ± 0·0027***	0·0297 ± 0·0068**
'Occupation time' (log days)	1·713	1·713	−0·182 ± 0·051**	−0·386 ± 0·087***

** Significant at $0·001 < P < 0·01$. *** 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 A tended to pass their meconium at a slower rate than those given milk B but, owing to the high variance, the difference was not significant.

Abomasal contents

The curd:whey ratio of the abomasal contents tended to be greater and the dry-matter content of the curd was significantly greater in calves given milk B than in those given milk A (Table 2). The addition of calcium increased the curd:whey ratio by a similar amount with both diets although, owing to the high variance, neither increase was significant.

DISCUSSION

Certain aspects of the findings reported here are in concordance with those in our earlier studies. Thus we have confirmed that when young calves are kept under conditions of high 'infection', heat treatment of the diet appears to predispose them to a high incidence of scouring and mortality associated with a localized intestinal infection with *E. coli*: under conditions in which little or no scouring occurs, there is a marked depression in weight gain of calves given the severely heated milks.

The results show also that, when calcium was added to the severely heated milks in an attempt to replace the loss in soluble and ionizable calcium that is known to occur as a result of heat treatment, there was no improvement in the incidence of scouring and mortality or in weight gains during the first 3 weeks of life. Our observations on the in vitro clotting properties of the spray-dried milk are in agreement with the more exact studies on the physical properties of the rennet curd made by G. W. Scott Blair & J. Burnett (1958, private communication) who found that the rigidity of the clot obtained with milk B ('mild' heat treatment) was greater than that obtained with milk A ('severe' heat treatment). It has also been observed that the addition of increasing amounts of calcium chloride to certain heated skim milks increases curd tension (Dill & Roberts, 1959) and reduces the time taken for rigidity of the curd to develop (Blair & Burnett, 1958). That the amount of calcium added to milk A in our experiments was more than adequate to restore the in vitro clotting properties of this milk to normal has been confirmed subsequently by J. C. Oosthuizen (1960, private communication).

Despite these findings, we have been unable to relate the physical appearance of the curd formed in the abomasum of the calf at 3 weeks of age to the degree of heat treatment imposed on the skim milks or to the addition of calcium, except that the dry-matter content of the curd of milk A was significantly lower than that of milk B; this finding may have been due to the greater quantity of whey retained in the curd of milk A. Although the addition of calcium tended to increase the amount of curd present in the abomasum irrespective of the skim milk the calf had been given, in no instance was the amount of abomasal curd in calves given no additional calcium considered to be subnormal. The disparity between the in vitro and in vivo observations could be explained if the pepsin–HCl enzyme system were not well developed during early postnatal life but if by 3 weeks of age secretion were sufficient to cause coagulation of the milk. This possibility was considered earlier by Kastelic, Bentley & Phillips (1950) who found that a synthetic milk containing 1·26 g calcium/l. was a satisfactory diet for the newborn calf, but that, when the concentration of calcium was reduced to 0·73 g/l., the milk did not clot with rennet and diarrhoea and subnormal growth resulted; this latter diet did not, however, produce such ill effects in the 2-week-old calf. Our findings appear to agree with this hypothesis. Thus the reduction in weight gains of calves given the 'severely' heated spray-dried milk was more marked during the first 12 days of life than later. Moreover, supplementation of this milk with calcium, although not having a demonstrable effect on weight gains over the entire 3-week period, appeared to have some beneficial effect during the first 12 days of life, whereas during this time an increase of much less magnitude was obtained from the addition of calcium to the 'mildly' heated milk.

There is little published work on the proteolytic activity in the abomasum during early postnatal life of the calf. Berridge, Davis, Kon, Kon & Spratling (1942–4) concluded that the milk-fed calf secretes only rennin and that the secretion of pepsin is not marked until the calf eats solid food. Dollar (1958) considered it was clear that the calf is poorly endowed with proteolytic digestive enzymes during the first few weeks of life and that rennin could account for almost all the proteolytic activity present. More recent work by Henschel, Hill & Porter (1961) has shown that although the 1- to 2-week-old calf secretes little or no pepsin, by 4 weeks of age, secretion of both rennin and pepsin occurs. However, further studies (J. W. G. Porter, personal communication) have shown that the young calf may secrete either rennin or pepsin or both and that the exact pattern of secretion is not predictable from the age of the animal or the nature of its diet.

It was shown by Hill (1956) that in the lamb relatively few parietal cells were present in the abomasum at birth, but by 36 h of life the number of cells had increased so that a pH value of just over 3·0 was attained. Similarly Pierce (1962) has found with the newborn calf after suckling that the pH of the abomasum contents did not reach a sufficiently low level for effective peptic proteolysis. Although we do not know the pH values of the abomasal contents of calves during the critical 1st week of life, by 7 days of age the pH of the abomasal contents ½ h after a meal has been found to fall to about 2·5 (J. W. G. Porter, personal communication). These findings suggest that the hydrochloric acid produced in the abomasum of the calf might be sufficient to clot severely heat-treated milks during the early postnatal period but from in vitro tests it is clear that a considerably larger quantity of acid will be required to clot these milks than would be necessary in the presence of adequate pepsin, and even then only a flocculent precipitate rather than a curd is formed.

The abomasal clot, by retaining nutrients for digestion by the relatively small amounts of proteolytic enzymes present in the abomasum, has long been known to be an essential feature of food utilization during early life (Mayer, 1935). If the clotting property of milk is impaired, there is a danger that incompletely digested milk may pass to the small intestine and there form a suitable substrate for the rapid multiplication of bacteria. Under conditions in which virulent strains of *E. coli* are present in the environment and when calves are given only small amounts of colostrum so that the initial clot in the abomasum is small, the apparent effect could well be a high incidence of scouring and mortality. Under conditions of low 'infection' and when larger amounts of colostrum are given, the loss of nutrients to the calf may be reflected in smaller live-weight gains during early life.

SUMMARY

1. Eighty-two newborn calves were used in two experiments to find the effect on performance during the first 3 weeks of life of adding calcium (500 mg/l. diet) to certain heat-treated milks. Experiments in vitro by ourselves and others have shown that heat treatment of milk may impair its rennet-clotting properties.

2. In the first experiment, made under conditions of high 'infection', the addition of calcium had no effect on the incidence of scouring and mortality in calves given diets containing (1) a spray-dried milk that had been 'severely' preheated (74° for about 30 min) during manufacture, (2) a UHT-treated (135° for 1–3 sec) separated milk or (3) raw separated milk.

3. In the second experiment, made under conditions of low to moderate 'infection', the addition of calcium had no effect on the incidence of scouring or on live-weight gains during the first 3 weeks of life of calves given diets containing spray-dried skim-milk that had been manufactured with either a 'severe' (74° for about 30 min, milk A) or a 'mild' (77° for 15 sec, milk B) preheating treatment. Addition of calcium to milk A appeared to have a slight beneficial though not significant effect on weight gains during the first 12 days of life.

4. Live-weight gains of calves given milk A were significantly less than those of calves given milk B, the difference being most apparent during early life. This finding confirms an earlier one.

5. The amount and consistency of the abomasal clot at the end of the experimental period appeared to be normal in calves given milk A; however the curd:whey ratio of the abomasal contents tended to be less and the dry-matter content of the curd was significantly less than in calves given milk B. The addition of calcium tended to increase the curd:whey ratio irrespective of the basal diet.

6. The hypothesis is discussed that impairment of the clotting properties of the diet given during early postnatal life may be associated with a lowered performance of the calf.

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